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East Europe Report

SCIENCE AND TECHNOLOGY



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EAST EUROPE REPORT
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INTERNATIONAL AFFAIRS

GDR-USSR JOINT TRAINING OF ELECTRICAL ENGINEERS REVIEWED

East Berlin PRESSE-INFORMATIONEN in German No 66, 11 Jun 85 pp 4-5

[Article by Siegfried Foerster, department head, Ministry for Electrical Engineering and Electronics: "GDR and USSR Jointly Train Specialists"]

[Text] As everywhere else in our republic, also in our industrial sector the stable and dynamic development resulting in today's efficiency is due to the close collaboration with the USSR. Based on nine governmental and eleven ministerial accords, close, fruitful scientific-technological co-operation in electrical engineering and electronics is under way with our partnership ministries in the USSR. It is above all concentrated on the main trends of electrical engineering, microelectronics, scientific instrument development, computer technology, precision mechanics and optics, and communications technology.

Imperative for continued good results in our cooperation is to prepare the personnel in good time for the higher tasks of the future, systematically to improve the level of political and technical training, and to perfect knowledge and skills. Training and continuing education in the Soviet Union offer fine opportunities for it. Learning from the Soviet Union has long been traditional for the revolutionary German workers class.

When based on the government accord on scientific-technological cooperation of August 1951, the first citizens of our country in the fall of 1951 began studying in the Soviet Union, a new chapter opened in the rich aid and support the USSR has unselfishly been granting us. Since then an average of 50 apprentices in the industrial sector, high school graduates with vocational training, have been delegated for a one-year preparatory course for foreign studies. Then they get the chance to study for 5 years at a USSR college in a specialty that is important to electrical engineering in the GDR.

More than 1,200 cadres who completed their studies, assistantships and supplementary studies in the USSR are working today in the industrial sectors of electrical engineering and electronics. More than half of them are assigned in the R&D sectors in the combines, enterprises and industrial institutes. They especially deal with tasks to be resolved through close cooperation with the Soviet Union and the other socialist states. With it, graduates of Soviet colleges often initiate the establishing and consolidation of close ties with enterprises and facilities in the USSR.

Graduates of Soviet colleges are heavily assigned to combines such as Robotron and those in microelectronics and automation plant construction and Carl Zeiss Jena. Those combines purposefully delegate young workers for foreign studies in the USSR. Through the training and continuing education of specialists in the USSR we are developing cadres who from their own experience are in close friendship linked with the Soviet Union and, after having completed their studies, engage in active public efforts.

More than 25 percent of the graduates of Soviet educational institutions are engaged in executive functions in the combines, enterprises and science institutions in the industrial sector. To raise this proportion further, for example, promotional accords are being concluded for technical and social advanced training and for providing skills in assuming managerial and specialized functions.

Longtime, diversified and fruitful cooperation has gone on in the students exchange between GDR and USSR educational institutions in electrical engineering and electronics. Stable partnership relations between educational institutes have developed, e.g. between the engineering school for machine construction and electrical engineering in Berlin-Lichtenberg and the Moscow electromechanical technical college, between the Friedrich Engels engineering school for electrical engineering and data processing in Goerlitz and the Leningrad Radiopolytechnicum, between the Hans Guenther engineering school for electrical engineering in Velten-Hohenschoepping and the Saporozhye industrial technicum, or between the Eisleben engineering school for electrical engineering and machine construction and the Shmel-nitski electromechanical technicum.

Way back in 1976 already, a friendship and cooperation accord was signed between the directors of the Novocherkassk electromechanical technicum and the Glashuette engineering school for precision mechanics. This year delegations between these educational institutions are being exchanged for the eighth time. From a practical year spent in the Soviet Union last year by 13 students and two specialized teachers, the partners in Glashuette gathered valuable experiences for their future assignments.

Soviet students in graduate studies at the clockworks of Glashuette become familiar with selected technologies and production organization and perfect their skills during that practical year. The demographic part of the practical year includes sightseeing in our republic and excursions to historical landmarks and memorials.

To meet, through well prepared cadres, the growing requirements of socialist economic integration, we shall continue delegating in the future young workers with best achievements in vocational training for studies in the USSR.

5885
CSO: 2302/110

HUNGARY

ADVANCED PROCESS FOR METAL GLASS FAILS INDUSTRIAL ADOPTION

Budapest MAGYAR HIRLAP in Hungarian 22 Aug 85 p 7

[Article by Marton Szluka: "Career of Metal Glass? As if Science Stopped at its Industrial Adoption; Inventors Disheartened"]

[Text] We can invest almost anything in our laboratories. Or, even if at some distance, we can follow the world. We are, however, extremely clumsy when it comes to the application of our results to practice. Here, for example, is the case of metal glass. Is it really inevitable that after 10 years a product meant for the market cannot leave the laboratory and is spoken of only as a possibility in the field of applications?

Janos Takacs, a technologist of the metal research group of the Central Physics Research Institute [KFKI] is angry. His 5-minute improvised demonstration, during which he produced a metal ribbon without a crystal structure from molten steel by supervast cooling (at the rate of 5,000,000 degrees per second), has not been successful this time. The snake-like ribbon that is thinner than a hair has burned to ashes at the end of the experiment, as if it were saying to its creator: This is how inventors' dreams are turned into nothing.

Although the unsuccessful experiment is not at all typical these days, it still has symbolic significance for me. It is a fact that the KFKI has had for many years a process that can be considered pioneering in the world. According to some, the preparation of such a new material is not an important scientific result. They are wrong, and the Institute has recognized the merits of the Hungarian pioneers of the superfast cooling technology--Csaba Hargitai, Gyula Kovacs, Antal Lovas and Janos Takacs--with the Janossy Prize.

On Home Waters

Fast cooling was already used in the 1960's at the California Institute of Technology for making experimental samples of materials. We succeeded in mastering the technology and tried to develop an industrial process. In the KFKI the first prototype machine was built. The starting material that is needed by industry can be made

on a laboratory scale. This can be used in many areas, from electronics to the construction and health fields. The Csepel Metal Works has from the beginning shown interest in the project, supported the research with money, and a few years ago, using the experiences of the KFKI it set up its own experimental machinery. This year at the BNV [National Trade Fair in Budapest] they have presented their first metal glass products.

Well, but... Putting things into practice is full of pitfalls, especially in Hungary. For a new material to be competitive in the marketplace it is not enough for it to have good technical parameters, it must also be competitive pricewise. That requires that it be manufactured in large amounts. As long as small quantities are made, the required investment is high and the new product cannot compete with the traditional soft-magnetic materials.

Worldwide, the application of metal glass ribbons started at the end of the 1970's in electric technology and in electronics. Transformers and other important components can be made from it. In Hungary applications are seriously hindered by the separation of the manufacture of basic materials and of finished components. The companies making basic materials make no complete transformers and the transformer manufacturers also like to sail on their home waters only.

In the developed industrial states on the other hand, companies deliver materials for transformers as well as completed units. It is true that even abroad many technical problems must be solved before the industrial use of a new material becomes satisfactory, but in the innovative chain reaching from research to final use the links are connected faster and more organically.

At Siemens for example, basic research was immediately followed by production in a large series. The idea was to provide physicists with an opportunity to examine the process continuously, so that the technology can be perfected while in contact with the developers. There is great need for this, because as is well known, there are great differences between laboratory production of individual units and the conditions needed for wide scale industrial processes.

Apparent Solution

Compared to the ideal procedure, the opportunities of the KFKI are limited--this is nothing surprising. We constantly run into the problem of the missing link in the chain. The institutionalized separation of research and manufacturing is a major stumbling block. The money is not enough either, but Janos Takacs does not consider that to be the major problem.

The Academy, the OMFB [National Technical Development Committee] and industry have had a part in the financing. In the first place, we would need talented technologists and machine constructors. We cannot find these, partly because we are working with a novelty and partly because there is a hiring freeze.

Of course, the industrial use of research results is not merely a question of the lack of a few experts or of money. It is already a kind of achievement that the solid state research laboratory of the KFKI which conducts basic research has come up with a product meant for sale in the marketplace. But as interesting as the new achievement is, it is disheartening to see what has happened to it. It looks like our knowhow has stopped at the introductory step. Whose task, after all, is the preparation of manufacture, the appraisal of the market, the fast training of specialists, the computation of profitability and who knows what else? In the West there is a distinct service sector doing these things while here, for lack of alternatives, the researchers themselves have to do it. They have created an economic group within their organization and as usual, have started going from door to door. Not totally unsuccessfully, but are such makeshift solutions suitable for remaining competitive?

From the metal glass ribbons we make a transformer that is used by the dynamic pickup system of high performance record players and studio equipment, says Janos Takacs. Our product was tested by Hi-Fi Magazine. Their results indicated that the parameters of our product have reached the developed western level. We have negotiated with the Ramovill company, saying we would deliver a few pieces.

This transformer is currently not on the Hungarian market. It is expensive, about DM800 a piece, with a pickup insert, even DM1000. In the past we have bought such transformers from the Ortofon Company but now we don't have the necessary foreign exchange. The transformer from the KFKI would be an order of magnitude cheaper and we would not need to import it.

Risk Analysis

This achievement is really applicable only to a small segment of the market, but even for this, one needs further investigations, negotiations, and risk analysis. The Hungarian career of metal glass has arrived here but we cannot stop and must ask the question: Where would development be if the conditions were right? Why is it that in Hungary the road to manufacture is constructed slowly and clumsily?

The academician Janos Bognar writes someplace that even if our market size is small, our capital is insufficient, our decision and execution processes are slow and the technique of profitable investments is underdeveloped here, we still cannot afford not to utilize research results developed in this country. Here or abroad, but somehow we must find a way to allow them to succeed. One condition for this is our participation in international technology transfer.

The "anticareer" of metal glass really is due to the lack of infrastructure serving science and industry, and the situation is similar with many other useful research results. As long as the infrastructure is not developed and the research results are applied only as an afterthought, inventors will remain disheartened, the economy will suffer, newspaper reporters will be angry and readers will be angry.

12846
CSO: 2502/69

POLAND

ADVANCES IN RADIobiological RESEARCH

Hypoxic Irradiation Effects

Warsaw NUKLEONIKA in English No 7-8, 1982 (signed to press Jan 85) pp 377-386

[Article by A.M. Dancewicz and M.M. Jelenska, Department of Radiobiology and Health Protection, Institute of Nuclear Research, 03-195 Warsaw, Poland; paper received April 1982; accepted October 1982]

[Text] The effect of hypoxic (10% of oxygen) conditions during irradiation of rats with 9.0 Gy dose of x-rays on determined in lung homogenate concentration of DNA, protein and collagen (as hydroxyproline) and on the activity of cathepsin, β -glucuronidase, acid phosphatase and plasminogen activator was studied. Assays were made from 1 day to 15 months after exposure. It was found that regardless of hypoxic conditions of irradiation, collagen content in rat lungs was increasing with time after exposure. However, hypoxic conditions resulted in partial decrease during later periods after exposure of other biochemical indices of irradiation. It was particularly evident in the case of the fibrinolytic activity which in hypoxic rats was increased during first several days only while in rats irradiated under normal conditions this increase persisted for more than one year after exposure.

Introduction

Partial-body or whole-body irradiation with sublethal doses of ionizing radiation often results in fibrotic changes late after exposure. During the latency period many biochemical and cellular effects of irradiation arise which may be involved to some extent as prerequisite of the prefibrotic stages. The most important of these seem to be the ones resulting in an accumulation of collagen and a rearrangement of its structural architecture in the intercellular matrix of connective tissue.¹ Severe fibrotic lesions are often fatal, and progressive fibrosis is considered as an incurable disease. Therefore, various attempts have been undertaken to influence and to reverse accumulation of fibrotic connective tissue. Application of inhibitors and drugs interfering with intra- and extra-cellular stages of collagen synthesis presents a promising possibility, scarcely explored so far.² In radiation-induced lung fibrosis

in rats colchicine applied 3 months after exposure of rat lung decreases to some extent accumulation of collagen.³ Beta-aminopropionitrile, a compound interfering with crosslinking of collagen, also prevents accumulation of insoluble collagen, when included into diet of irradiated rats (⁴, G.B. Gerber, unpublished). Temporary intraarterial blockage by degradable microspheres results in local hypoxia which significantly decreases the frequency of fibrotic lesion in rat gut.⁵ The protective effect of a diminished oxygen tension in the irradiated object has been known for a long time and hypoxic conditions have been successfully applied in selective protection of normal tissues by irradiation of tumor bearing mice,⁶ and were also tried with some success in radiotherapy in humans (Yarmonenko, private communication). However, it seems that the effect of hypoxia on the development of late radiation damage has attracted little attention of those studying modification of radiosensitivity of biological objects by oxygen tension.

Some biochemical changes in animals surviving the acute phase of the radiation insult last for a long time. This has been shown by others (for review see ¹) and by our group in lung tissue of rats.^{7,8} In this paper we present data on biochemical parameters assayed in lungs of rats exposed to irradiation under hypoxic conditions.

Experimental

Mature male rats of Wistar strain were irradiated in a rectangular plexiglas box separated into three compartments for individual animals. The box was connected to a chamber in which nitrogen was added to air to diminish the oxygen content in the breathing mixture to 8-10 percent. The animals were kept under hypoxic condition for 10 minutes before and during irradiation. The animals were exposed to a total dose of 9 Gy at the dose rate of 25 cGy·min⁻¹ of x-rays generated in a Stabilipan apparatus at 180 kV and 18 mA and filtered through 1 mm Cu. Full scatter of radiation was achieved by placing the box on a 6 cm thick preswood plate and lining the sides of the box with pieces of wood. Control animals were kept for 45 minutes under similar conditions, i.e. in the same cage and breathing the hypoxic mixture. Subsequently, the irradiated and control rats were kept in animal quarters under standard conditions until the time of the scheduled sacrifice.

Rats were sacrificed under ether anesthesia by cutting the spinal cord. The chest was opened and the lungs were washed through the trachea with 5 ml of saline three times. Then the lungs were excised, freed from larger airways, weighted and homogenized in water.

The following biochemical analyses were carried out in the lung homogenate: protein by the biuret method,⁹ activity of acid phosphatase and beta-glucuronidase by measuring spectrophotometrically phenolphthalein released from the respective substrates,¹⁰ activity of cathepsin D by fluorometric assay of naphtylamine liberated from Na-benzoyl-dL-arginine-beta-naphtyl amide after 18 h incubation.¹¹ DNA and collagen were isolated by extracting a perchloric acid precipitate of the homogenate with the 5 percent trichloroacetic acid.⁷ DNA concentration was determined by the diphenylamine reaction,¹² collagen by the method of Stegemann and Stadler.¹³

In order to assay plasminogen activator, a lung tissue sample was homogenized for 3 minutes in 0.35 M phosphate buffer, pH 7.5 containing 0.1 percent Tween 80 then centrifuged (20 min at 12,000 x g). The supernatant was dialysed against 0.05 M tris-HCl buffer, pH 8.0 containing 0.005 percent of Tween 80, centrifuged (10 min at 10,000 x g) and the activity of plasminogen activator was determined. The assay system composed of 0.05 ml of the extract, 0.45 ml of 0.05 M tris-HCl buffer, pH 8.0 containing 0.005 percent Tween 80 and 0.10 ml of plasminogen (5 mg/ml) was preincubated at 37°C for 30 min. Chromozym TH (Tosyl-L-glycyl-L-prolyl-L-arginyl-p-nitroanilide HCl, product of Boeringer, Mannheim) 0.20 ml of 2 mM, a substrate for plasmin was added followed after 2 minutes incubation at 37°C by 0.20 ml of conc. acetic acid. The concentration of p-nitroaniline released by the generated plasmin from the substrate was measured spectrophotometrically at 405 nm.

Results

The post-irradiation changes in the various biochemical parameters in lung tissue of rats irradiated with a dose of 9 Gy of x-rays under hypoxic conditions are presented in Tables 1 and 2 and in Figure 1. These changes are given in relation to values obtained for control animals (hypoxic, sham irradiated). For the evaluation of the effect of hypoxia on the radiation induced changes in rat lungs the data reported here were compared with the ones obtained in our earlier study of radiation induced changes in biochemistry of lung tissue of whole-body irradiated rats (Table 3).⁸

Protein content in lung tissue was less affected by hypoxia and irradiation than by irradiation alone. With the exception of 1 week after exposure there was no significant decrease in protein concentration. On the other hand hypoxic conditions did not influence accumulation of collagen in lungs of irradiated rats. Collagen accumulation during the latter stages of fibrotic development was almost identical in both hypoxic and nonhypoxic rats exposed to x-rays. Cathepsin activity which has been found increased significantly in lungs of whole-body irradiated rats seems to be normalized at the later post-irradiation periods (5-12 months) in rats irradiated under hypoxic conditions. No significant differences were found in the activity of beta-glucuronidase in lung tissue irrespectively of the conditions of irradiation and thus is also true for activity of acid phosphatase. Irradiation caused decrease of the activity of this enzyme both in normal and in hypoxic rats, especially during the later stages of fibrotic development. Changes in DNA concentration in lungs of rats irradiated under hypoxic conditions do not differ significantly--with two exceptions--from those obtained for sham irradiated hypoxic rats. During the first days after exposure, DNA concentration was decreased and two months after irradiation it was increased. Fibrinolysis was studied using two different methods. In the present study, the activity of plasminogen activator in the extracts from lung tissue was assayed while in the previous work⁸ the final effect of fibrinolysis (lysis of fibrin plate) was measured. The plasminogen activator in lung tissue of hypoxic rats was elevated during the first week after exposure. Thereafter the activity of the activator returned to a level comparable to that found in nonirradiated rats (Figure 1). In rats irradiated in nonhypoxic conditions the activity of fibrinolysis remained at the elevated level up to one year after exposure (i.e. to the end of experiment).

Table 1. DNA, Protein and Collagen (as hydroxyproline) Content in Lungs of Rats Irradiated With 9.0 Gy Dose of α -rays Under Hypoxic Conditions

Time after irradiation	Number of animals	DNA mg/g tissue	P	Protein mg/g tissue	P	Hydroxyproline $\mu\text{g}/\text{mg}$ protein	P
0 (sham irr.)	26	1.73 ± 0.49	—	203.8 ± 48.2	—	6.19 ± 1.77	—
1 day	5	1.34 ± 0.07	0.05	201.0 ± 13.0	NS	5.31 ± 0.98	NS
2 days	5	0.90 ± 0.08	0.001	183.6 ± 11.0	NS	6.40 ± 0.70	NS
1 week	5	1.64 ± 0.39	NS	230.1 ± 38.0	NS	7.37 ± 1.19	NS
2 weeks	5	1.40 ± 0.08	NS	158.9 ± 10.0	0.05	5.29 ± 0.53	NS
2 months	5	2.11 ± 0.12	.05	165.6 ± 7.0	0.05	8.58 ± 1.27	0.05
3 months	6	2.02 ± 0.33	NS	186.6 ± 17.0	NS	10.07 ± 2.53	0.001
5 months	5	1.45 ± 0.21	NS	183.7 ± 5.0	NS	6.51 ± 1.25	NS
12-15 months	19	1.89 ± 0.47	NS	213.7 ± 54.5	NS	8.59 ± 3.21	0.05

Concentration of DNA was determined by the diphenylamine method,¹² concentration of protein by the Biuret method⁹ and hydroxyproline by Stegemann and Stadler method.¹³ Statistical analysis of the significance of changes between irradiated hypoxic and hypoxic sham irradiated rats was calculated according to the Student test. N.S. stands for not significant.

Table 2. The Activity of Hydrolytic Enzyme in Lung Homogenate of Rats Irradiated With 9.0 Gy of X-rays Under Hypoxic Conditions (10 percent oxygen)

Time after irradiation	Number of	Acid phosphatase A_{546} mg/tissue	P	B-glucuronidase A_{546} mg tissue	P	Cathepsin D F_{450} mg tissue	P
0 (sham irr.)	26	0.222 ± 0.07	—	0.133 ± 0.02	—	14.25 ± 3.5	—
1 day	5	0.242 ± 0.05	NS	0.159 ± 0.06	NS	20.57 ± 7.3	0.01
2 days	5	0.190 ± 0.02	NS	0.100 ± 0.02	0.05	16.86 ± 3.7	0.10
1 week	5	0.180 ± 0.03	NS	0.092 ± 0.02	0.01	10.56 ± 3.2	0.05
2 weeks	5	0.164 ± 0.03	0.05	0.096 ± 0.02	0.01	20.03 ± 3.5	0.01
2 months	5	0.140 ± 0.04	0.01	0.123 ± 0.02	NS	16.66 ± 1.9	0.1
3 months	6	0.168 ± 0.04	0.05	0.126 ± 0.02	NS	18.88 ± 4.6	0.01
5 months	5	0.102 ± 0.01	0.01	0.126 ± 0.03	NS	15.36 ± 3.4	NS
12-15 months	19	0.182 ± 0.05	0.05	0.142 ± 0.03	NS	11.38 ± 2.1	0.05

The values are expressed as the mean ± S.D. of the net increase in absorption (A_{546}) or fluorescence (F_{450}) per mg of wet tissue. P values for statistical significance of the difference between irradiated and sham irradiated animals are given, NS stands for not significant.

Table 3. Comparison of Biochemical Changes in Lungs of Rats Irradiated Under Hypoxia (H) with 9.0 Gy Dose of X-rays and Under Normal Conditions (N) With a Dose of 6.5 Gy of X-rays. The mean \pm S.D. values are given as percent of the values obtained for sham irradiated controls. Groups differing significantly are indicated with asterisks: * $P \leq 0.05$; ** $P \leq 0.01$; — data not available.

		1	2	8-12	20-24	56 and over
		Weeks after exposure				
Protein	N	83 \pm 8	** 115 \pm 9	** 83 \pm 6	* 75 \pm 6	** 81 \pm 1,777 **
	H	113 \pm 6	77 \pm 6	91 \pm 9	90 \pm 3	110 \pm 25
Collagen	N	—	—	104 \pm 2	** 113 \pm 12	142 \pm 11
	H	119 \pm 16	85 \pm 10	138 \pm 15	105 \pm 19	139 \pm 31
Fibrinolysis	N	135 \pm 26	170 \pm 8	** 129 \pm 18	** 120 \pm 14 **	131 \pm 17 **
	H	156 \pm 5	95 \pm 13	102 \pm 88	105 \pm 13	105 \pm 11
Cathepsin	N	123 \pm 31	** 147 \pm 10	129 \pm 23	170 \pm 20 **	123 \pm 30 **
	H	74 \pm 30	140 \pm 17	132 \pm 24	108 \pm 22	80 \pm 18
Beta-glucuronidase	N	70 \pm 26	72 \pm 15	102 \pm 20	102 \pm 11	118 \pm 21 *
	H	69 \pm 2	72 \pm 2	95 \pm 2	95 \pm 3	107 \pm 2
Acid phosphatase	N	41 \pm 36 *	172 \pm 14 **	90 \pm 33	67 \pm 23	66 \pm 51 **
	H	81 \pm 2	72 \pm 2	76 \pm 3	46 \pm 1	82 \pm 3

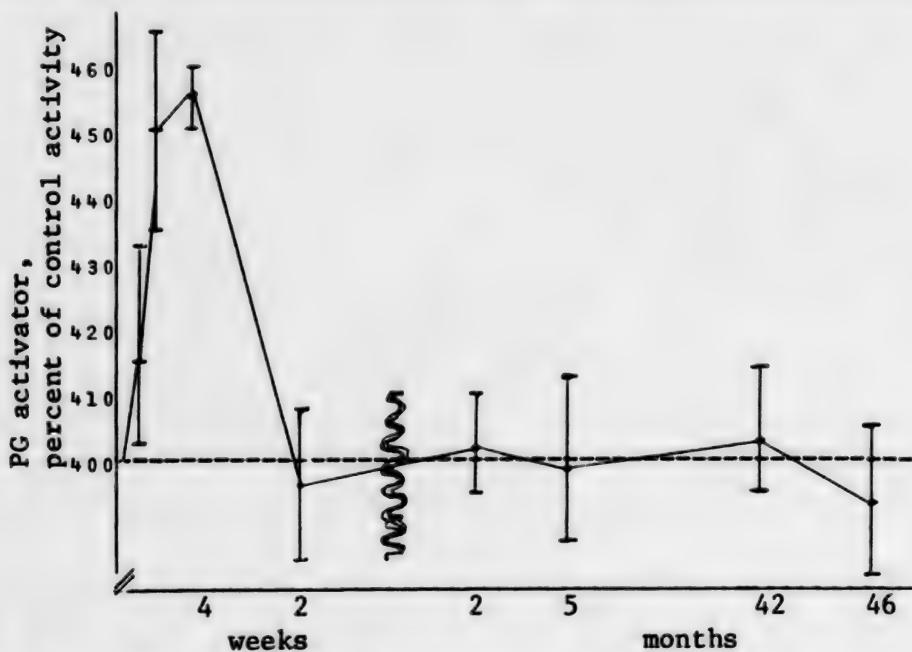


Figure 1. Effect of Whole Body Irradiation With 9.0 Gy Under Hypoxia on Plasminogen Activator Activity in Rat Lung Extracts

Discussion

Hypoxic rats survive larger doses of ionizing radiation than rats irradiated under aerobic conditions. Hypoxic conditions as applied in this experiment allowed to increase the radiation dose to 9 Gy at which the overall survival rate of irradiated rats was about 50 percent, i.e. the same as for rats irradiated under aerobic conditions with a dose of 6.5 Gy which was applied in our earlier study.³ This experiment thus compares damage in lung at comparable degrees of acute generalized damage of whole-body irradiated rats. The data presented demonstrate, however, that under such conditions hypoxia during irradiation does not modify the development of the late effects resulting eventually in lung connective tissue fibrosis. Comparison of the radiation-induced accumulation of collagen in hypoxic and in aerobic rats (Table 3) shows no difference. This may indicate that hypoxia during irradiation does not hinder the development of lung fibrosis. This conclusion should, however, be confirmed by histological examination to prove whether accumulation of collagen in this case is associated with the fibrotic transformation of connective tissue.

Hypoxia does, however, reduce the development of other biochemical indices of late effects in the lung of rats in comparison to aerobic rats in spite of the fact that the radiation dose applied to hypoxic rats was 1.4 times higher than the one used for aerobic rats. In hypoxic rats, catheptic activity of the lung homogenate elevated at earlier stages returned to normal values five months after exposure. Fibrinolysis was elevated even for a shorter period of time. Already two weeks after exposure the activity of plasminogen activator was at its normal level in lung extracts of hypoxic rats while it remained elevated to the end of experiment (12 months after exposure) in rats irradiated under aerobic conditions. Although in both experimental groups fibrinolysis was assayed by a different technique both methods gave similar results when checked repeatedly in different lung changes in fibrinolysis depends largely on the radiation-induced changes in activity of plasminogen activator.

Concentration of DNA in lungs of irradiated hypoxic rats was decreased during the first days after exposure and increased at two months later. Compared to nonirradiated hypoxic controls it reflects perhaps the cell depletion immediately after exposure and the invasion or multiplication of cells preceding or coinciding with the increase in synthesis of collagen 2-3 months thereafter. Changes in DNA content and thus in cellularity of lungs irradiated under hypoxia were much smaller than those observed following high level local, hemithorax irradiation.⁷

The other parameters studied showed no significant difference between rats irradiated under hypoxia and in air. In general, it can be concluded that hypoxia reduces to some extent certain biochemical sequelae but probably does not prevent the development of lung fibrosis.

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Tritium Ingestion in Rats

Warsaw NUKLEONIKA in English Vol 27 No 7-8, 1982 (signed to press Jan 85)
pp 389-403

[Article by Zofia Pietrzak-Flis and Irena Radwan, Central Laboratory for Radiological Protection, 03-154 Warsaw, Poland; paper received January 1982, accepted April 1982]

[Text] An effect of chronic ingestion of tritiated water and tritiated food on growth and reproduction of Wistar rats was evaluated. The animals were exposed during one or three successive generations. Ingestion of tritiated water at activity of 185.0 and 370.0 kBq/ml or tritiated food at activity of 144.3 kBq/g affected growth of the first generation (F_1) rats. In groups exposed to tritiated water the effect was transient. Exposure to tritiated food at 48.1 kBq/g or to tritiated water at 37.0 kBq/ml affected the growth of F_2 generation rats only. A significant reduction in a relative testis weight was observed in a group exposed to tritiated water at activity of 370.0 kBq/ml, while sperm production was affected in all exposed groups. Ingestion of tritiated food caused higher reduction in sperm count than tritiated water. The effect of tritium on growth of rats and ability for sperm production depended on the absorbed dose and the form of ingested tritium.

Introduction

Tritium present in the environment enters animals and human body with water and with food, in which it is bound organically. There have been many studies concerning an effect of tritiated water (HTO) and tritiated organic compounds such as thymidine or uridine, or morphological and functional changes in laboratory animals.¹⁻⁷ However, no data have been found in the literature on the effect of tritium organically bound in food (T-food).

This study was undertaken to determine an effect of continuous exposure to tritiated water and tritium organically bound in food on growth and some reproductive functions of rats.

Materials and Methods

Two experiments with various doses of tritium were performed.

Experiment 1

Thirty-six virgin Wistar females (P_0), aged about three months, weighing on average 180 ± 10 g, were randomly divided into three groups, 12 animals each. The first group was exposed to tritiated water of activity 37.0 kBq/ml, the second group to tritiated food of activity 48.1 kBq/g, and the third one was a control group. Tritiated food consisted of freeze-dried rabbit meat and powdered standard chow. Tritiated meat was obtained by i.p. injections of rabbits with HTO in the amount of about 0.4 MBq/g of body weight every second day for 2 weeks, then rabbits were killed and their soft tissues were freeze-dried. The activity of dry meat was in the range of 0.3-0.4 MBq/g and distribution of tritium among the biochemical fractions was as follows: in proteins 49.9 ± 0.5 percent, in nucleic acids 19.2 ± 2.3 percent, in lipids 27.4 ± 2.9 percent of the total activity.

The animals of HTO and control groups were given uncontaminated freeze-dried rabbit meat mixed with powdered standard chow in the same proportion as in T-food group. The rats of all groups received food and water ad libitum.

The females were individually housed in metabolic cages within the enclosed glove boxes. The glove boxes were connected to a ventilation system to avoid an additional contamination of animals with inhaled air.

After 3 weeks of exposure the females were mated to nonexposed males of the same strain. The exposure was continued up to delivery of F_3 generation rats. Details of the experiment were presented in the previous work.⁸

Observations on F_1

F_1 litters were delivered naturally and the litter size was recorded. On the 3d day postnatally the rats were weighed and litters were normalized to eight pups. The growth of animals was assessed by weighing at the 7th, 10th, 14th, 18th, 21st, 35th and 100th day of age.

On the 21st and 60th days one male and one female of each litter were killed, and on the 120th day the males of all groups were killed. Liver, kidneys, brain, spleen, heart and lungs were removed and placed in tared vials for wet weight determination. Additionally the testis, epididymis and vas deferens of 60 and 120 day-old males were taken. Testis were weighed, the epididymis and vas deferens were minced in 1 ml of saline and diluted to 10 ml with neutral buffered formalin. The aliquots were taken for sperm counting with the use of a hemocytometer.

At the age of three months the females of F_1 were mated to nonlitter mate males of the same group.

Observations on F₂

The F₂ litters were delivered naturally. They were normalized and weighed similarly as F₁ rats. At 21 days of age one female of each litter was dissected and the same organs as in F₁ removed and weighed. At the age of about 3 months females were mated to nonlitter mate males of the same group.

Observations on F₃

The F₃ litters were delivered at full term by Caesarian section from dams which had commenced natural delivery. The ovaries and uterine contents were examined. The number of corpora lutea was counted and resorptions (early embryonic deaths) was evidenced by a dark "spot." The preimplantation deaths were assessed by the difference between the corpora lutea count and the number of implantation sites with live and resorbed fetuses. The litter size was recorded, the fetuses were dried and weighed. Two males and two females of each litter were dissected and liver, kidneys, lungs, brain and heart were taken and weighed.

Experiment 2

Forty eight virgin Wistar females were randomly divided into four groups, 12 animals each. The first group was maintained on tritiated food at activity of 144.3 kBq/g, prepared as in Experiment 1. Animals of the second and third groups ingested tritium in drinking water at activity of 185.0 and 370.0 kBq/ml, respectively. The fourth group was a control one.

The exposure of females was started 3 weeks before mating to nonexposed males of the same strain and continued to the 71st day of age of F₁.

The F₁ litters were delivered naturally, litter size was recorded, and on the 3d day postpartum litters were normalized to eight pups and weighed. The weight of animals was also recorded at similar intervals as in Experiment 1, ending on the 71st day.

On the 21st and 71st day one male and one female of each litter were dissected and the same organs as in Experiment 1 were taken.

Results

In this study three different levels of HTO in drinking water and two levels of tritium organically bound in food were applied. The relation of activity in groups exposed to HTO was 1:5:10, and in groups exposed to tritiated food it was 1:3.

The radiation doses absorbed in tissues of rats were calculated on the basis of tritium content in wet tissues, according to the method described by Patzer.⁹ Tritium content in wet tissue was estimated using tritium concentrations in dry tissue, in body water and water content in particular tissue. The radiation doses absorbed in four selected tissues of 21-day,

Table 1. Radiation Doses Absorbed in Tissues of F₁ 21 Day-old Animals (cGy), and Contribution of Nonexchangeable Tritium to the Total Doses (percent)

Tissue	kBq/ml HTO exposure			T-food exposure kBq/g		Contribution of NET (percent)	
	37.0 ^a	185.0	370.0	48.1 ^a	144.3 ^a	HTO groups	T-food groups
Liver	7.4±0.2 ^b	31.8±0.2	63.0±0.9	7.1±0.3	19.0±0.5	6.0-7.0	19.2-23.2
Brain	8.2±0.5	35.9±0.6	71.0±1.0	6.7±0.4	19.5±0.6	6.4-9.6	11.0-13.7
Ovaries	7.7±0.3	32.7±1.1	65.2±1.5	8.6±0.5	21.3±1.2	5.9-6.3	25.0-34.7
Testis	8.9±0.3	37.7±0.6	74.9±0.8	7.2±0.3	20.3±0.6	3.0-3.5	7.0-7.5

^aNote: animals of F₂ received the same radiation dose

^bNote: mean ± SE

Table 2. Radiation Doses Absorbed in Tissues of F₁ 60 and 71 Day-old Rats (cGy)

	HTO exposure, kBq/ml			T-food exposure, kBq/g	
	37.0 ^b	185.0 ^c	370.0 ^c	48.1 ^b	144.3 ^c
Liver	14.1 ± 0.4 ^a	68.8 ± 0.5	136.3 ± 1.9	13.4 ± 0.5	41.2 ± 1.1
Brain	15.6 ± 0.9	77.6 ± 1.3	153.5 ± 2.2	12.7 ± 0.8	42.2 ± 1.2
Ovaries	14.8 ± 0.7	70.8 ± 2.3	141.0 ± 3.3	16.4 ± 0.9	46.1 ± 2.7
Testis	16.9 ± 0.5	81.6 ± 1.2	162.0 ± 1.8	13.7 ± 0.5	44.0 ± 1.2

^aNote: mean ± SE

^bNote: 60 day-old rats

^cNote: 71 day-old rats

Table 3. Radiation Doses Absorbed in Tissues of F₁ 120 Day-old Males (cGy)

Tissue	HTO exposure 37.0 kBq/ml	T-food exposure 48.1 kBq/g
Liver	24.4 ± 0.7 ^a	23.3 ± 0.9
Brain	27.0 ± 1.6	22.0 ± 1.4
Testis	29.3 ± 0.9	23.7 ± 0.9

^aNote: ± SE

60-day and 71-day, and 120 day old rats are presented in Tables 1, 2 and 3, respectively. In Table 1 are also given the contributions of nonexchangeable tritium to the total doses.

The exposure of animals to the lowest level of ingested HTO or T-food (Experiment 1) did not affect body weight or relative organ weights of the F₁ generation of rats. However, slight decrease in body weight of rats was noticed in the F₂ generation. In rats of age up to 18 days the effect was more distinct than in older ones (Table 4). Also the relative weight of liver, kidneys and brain of the F₂ 21-day old females in exposed groups differed from the control, but the difference was not statistically significant (Table 5).

Table 4. Body Weights of F₂ Generation Males and Females (g)

Age days	Males			Females		
	Control	(6) ^a	(6)	(6)	(6)	(6)
3	6.3 - 0.4 ^b	5.8 ± 0.3	5.9 ± 0.4	5.8 ± 0.3	5.3 ± 0.4	5.4 ± 0.3
7	(6)	(6)	(6)	(6)	(6)	(6)
	10.6 ± 0.9	9.7 ± 0.7	9.3 ± 0.7	10.4 ± 0.9	9.2 ± 0.6	8.8 ± 0.7
10	(6)	(6)	(6)	(6)	(6)	(6)
	15.7 ± 1.3	14.0 ± 1.1	13.6 ± 1.1	14.8 ± 1.3	12.9 ± 1.2	13.5 ± 1.3
14	(6)	(6)	(6)	(6)	(6)	(6)
	20.3 ± 0.8	19.6 ± 1.3	20.0 ± 1.9	19.7 ± 1.4	18.4 ± 1.3	19.6 ± 1.7
18	(6)	(6)	(6)	(6)	(6)	(6)
	27.3 ± 1.0	25.1 ± 3.1	26.1 ± 2.0	25.4 ± 1.1	25.2 ± 2.1	26.0 ± 2.5
21	(6)	(6)	(6)	(6)	(6)	(6)
	31.3 ± 2.5	31.2 ± 2.9	31.1 ± 2.7	28.7 ± 2.0	28.2 ± 1.8	29.7 ± 2.6
35	(6)	(6)	(6)	(6)	(6)	(6)
	83.1 ± 4.6	73.4 ± 5.6	77.4 ± 6.7	73.1 ± 2.8	64.9 ± 1.0	69.4 ± 5.8
100	(6)	(7) ^c	(6) ^c			
	262.9 ± 5.2 ^c	242.7 ± 7.9	264.7 ± 5.2			

^aNote: number of litters

^bNote: mean of the litter means ± SE

^cNote: number of animals

At the higher tritium activities (Experiment 2) the influence of exposure on body weight was observed in the F₁ generation of rats (Tables 6 and 7). In the HTO groups the effect was transient, while in the group exposed to T-food the body weight was decreased throughout the entire experiment. The reduction in body weight was not accompanied by reduction in relative organ weights, with exception of testis of the 21 as well as 71-day old males.

Table 5. Relative Organ Weights of 21 Day-old Females of F₂ Generation

Tissue	Control	HTO group	T-food group
		37.0 kBq/ml	48.1 kBq/g
Liver	(7) ^a 3.92 ± 0.17 ^b	(9) 3.69 ± 0.07	(7) 3.70 ± 0.18
Kidney	(7) 1.21 ± 0.02	(9) 1.16 ± 0.03	(7) 1.13 ± 0.02
Lung	(7) 1.03 ± 0.05	(7) 1.06 ± 0.04	(6) 1.04 ± 0.07
Brain	(6) 4.61 ± 0.17	(8) 4.54 ± 0.16	(6) 4.43 ± 0.29
Heart	(7) 0.61 ± 0.02	(7) 0.59 ± 0.01	(7) 0.60 ± 0.02
Spleen	(6) 0.27 ± 0.01	(9) 0.27 ± 0.01	(7) 0.26 ± 0.01

^aNote: number of litters

^bNote: mean ± SE

Table 6. Body Weight of F₁ Generation Males (Experiment 2) (g)

Treatment group	Age, days						
	3	10	15	21	35	50	71
Control	(8) ^a 8.7±0.2 ^c	(10) 19.3±0.7	(10) 30.1±1.1	(10) 42.1±2.0	(9) 108.8±3.3	(9) 174.4±3.6	(7) 251.4±8.2
T-food 144.3 kBq/g	(7) 8.0±0.4	(10) 18.1±1.9	(9) 26.4±1.0	(10) 37.7±1.2	(10) 96.8±2.3	(10) 159.1±6.9	(8) 220.9±9.4
HTO 185 kBq/ml	(9) 8.1±0.3	(9) 19.0±0.4	(8) 27.9±0.7	(8) 38.7±1.7	(9) 103.3±3.8	(8) 182.7±7.0	(8) 267.1±12.5
HTO 370 kBq/ml	(5) 7.0±0.3 ^d	(8) 17.4±1.1	(7) 26.9±1.7	(7) 37.1±1.7	(7) 99.8±3.9	(8) 179.2±5.3	(6) 252.2±11.1

^aNote: number of litters

^bNote: number of rats

^cNote: mean ± SE

^dNote: p < 0.01

^eNote: p < 0.05

Table 7. Body Weight of F₁ Generation Females (Experiment) (g)

Treatment group	Age, days						
	3	10	15	21	35	50	71
Control	(8) ^a 8.0±0.3	(11) 18.3±0.7	(11) 29.6±1.0	(12) 39.0±1.7	(12) 90.0±2.3	(11) 132.8±2.6	(15) ^b 172.4±3.4
T-food 144.3 kBq/g	(7) 7.4±0.4	(9) 17.9±0.3	(9) 26.2±1.3	(10) 37.3±1.3	(10) 85.4±1.8	(10) 129.7±3.5	(8) 165.7±3.4
HTO 185.0 kBq/ml	(8) 7.4±0.3	(9) 17.7±0.7	(8) 26.0±0.5	(8) 36.2±1.2	(8) 86.1±2.2	(8) 135.1±3.9	(8) 177.2±3.2
HTO 370 kBq/ml	(5) 7.0±0.3	(8) 17.0±1.0	(8) 26.3±1.3	(7) 34.2±1.4	(8) 86.0±2.9	(8) 130.9±2.5	(8) 175.4±7.4

^aNote: number of litters^bNote: number of rats^cNote: mean ± SETable 8. Effects of Tritium on the Weight of Testis and Sperm Count of F₁ Rats

	Control	HTO kBq/ml exposure			T-food exposure kBq/g	
		37.0	185.0	370.0	48.1	144.3
Relative testis weight, percent 21 day-old	(6) ^a 0.44±0.02 ^b		(6) 0.41±0.01	(6) 0.33±0.01 ^c		(8) 0.42±0.01
60 day-old	(10) 1.44±0.03	(7) 1.48±0.04			(8) 1.38±0.01	
71 day-old	(9) 1.20±0.05		(8) 1.03±0.03	(7) 0.94±0.04 ^c		(7) 1.16±0.05
120 day-old	(8) 1.15±0.04	(8) 1.14±0.03			(9) 1.11±0.01	
Sperm count (number of epididymal sperm) sperm /g testis x 10 ⁷	(9) 66±4	(7) 59±5			(8) 59±8	
60 day-old	(10) 31±3		(8) 23±3	(7) 11±1 ^c		(8) 12±2 ^c
71 day-old	(10) 86±10	(8) 70±3			(8) 72±5	

^aNote: number of samples^bNote: mean ± SE^cNote: p < 0.1 by Dunnett's

The relative weight of testis and ability to produce the sperm, expressed by the number of sperm in epididymis and vas deferens per gram of testis, are presented in Table 8. In Experiment 1 with 37.0 kBq/g a small depression in sperm count was observed in the exposed groups. In Experiment 2, a reduction in sperm count was higher. Relative weight of testis was affected significantly in the group exposed to HTO in the concentration of 370.0 kBq/ml.

Effect of dose absorbed in testis on sperm production is shown in Figure 1. This figure gives a sperm count, expressed in percent of control groups. As can be seen from this figure, the ability for production of sperm decreased with the increasing dose. Using a least-squares analysis, the experimental data for males exposed to HTO were fitted, with correlation coefficient $r = 0.96$, to the linear equation:

$$y = 97.44 - 0.37 D$$

while data for males exposed to tritiated food were fitted with $r = 0.99$ to linear-quadratic equation:

$$y = 99.07 - 0.07 D - 0.03 D^2$$

where y is relative sperm count as absorbed dose D .

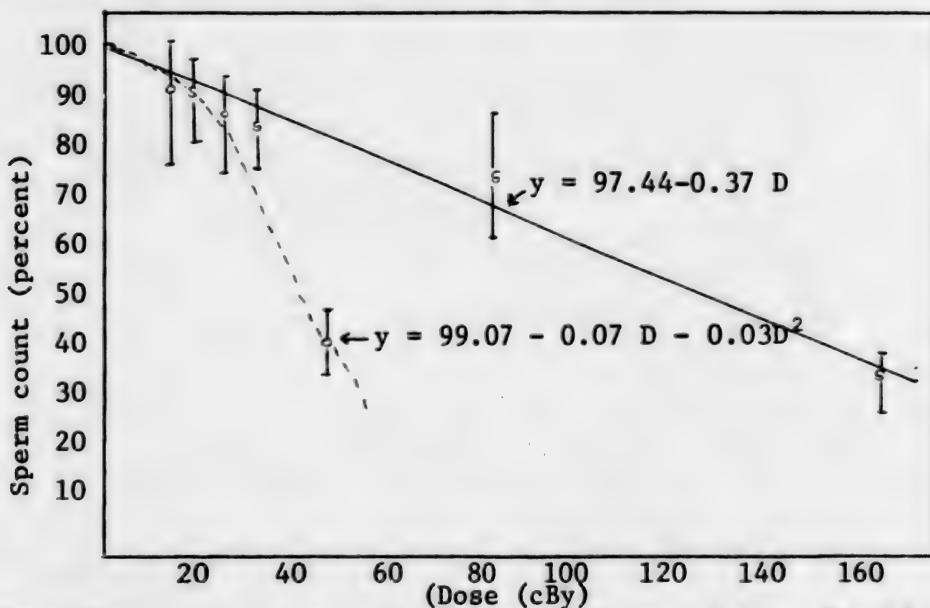


Figure 1. Sperm Count (in a percent of a control group) as a Function of Doses Absorbed in Testis After Exposure to Tritiated Water (—●—) or Tritium Organically Bound in Food (---□---).

Exposure of P_0 , F_1 and F_2 females had some effect on litter size. However, in none of these groups this effect was statistically significant (Table 9). The resorptions and ovulations were studied only in F_2 females. From the data presented in Table 9 it can be seen that in females exposed to tritiated food the resorptions and preimplantation deaths were slightly higher in comparison to the control and HTO groups.

Table 9. Effects of Tritium on Litter Size, Resorption, Ovulation and Preimplantation Death

	Control	HTO kBq/ml exposure			T-food exposure kBq/g	
		37.0	185.0	370.0	48.1	144.3
Litter size						
F ₁	(7) ^a 9.6±0.8 ^b	(8) 8.4±0.5			(7) 8.7±0.5	
F ₂	(8) 10.9±0.8	(10) 9.2±0.7			(8) 9.5±0.3	
F ₃	(7) 10.8±0.9	(8) 9.3±0.4			(7) 9.3±0.5	
F ₄	(12) 9.8±0.7	(11) 8.6±0.9	(11) 9.1±0.7			(11) 9.2±0.7
Resorptions						
Average percent per litter F ₃	(7) 7.8±4.0	(8) 7.0±2.1			(7) 11.2±5.7	
Ovulations						
Average per litter	(7) 12.6±0.7	(8) 11.4±0.8			(7) 13.8±1.0	
Preimplantation deaths						
Average per litter F ₃	(7) 1.3±0.7	(8) 1.4±0.6			(7) 3.7±0.7	

^aNote: number of litters

^bNote: mean ± SE

Exposure to tritiated water or to tritiated food in doses applied in the Experiment 1 did not affect body weight of F₃ males and females. Among the organs studied, there were reduced the relative weight of brain in animals exposed to tritiated water and relative liver weight in animals exposed to tritiated food. The relative weight of kidneys in rats of the tritiated food groups was significantly increased (Table 10).

Table 10. Relative Weight of F₃ Kidneys, Brain and Liver (in percent of body weight)

Tissue	Control	HTO 37.0 kBq/ml	T-food 48.1 kBq/g
Kidneys	(7) ^a 0.80 ± 0.002 ^b	(8) 0.84 ± 0.02	(6) 0.93 ± 0.02 ^c
Brain	(7) 4.27 ± 0.05	(8) 4.11 ± 0.08	(6) 4.23 ± 0.09
Liver	(7) 6.32 ± 0.30	(7) 6.11 ± 0.21	(6) 5.97 ± 0.35

^aNote: number of litters

^bNote: mean of litter means ± SE

^cNote: significantly different from control by Dunnett's t-test at p < 0.01

Discussion

Studies on succeeding generations of rats and mice exposed to tritiated water indicate that some cumulative effects of tritium exposure can exist.^{2,10} Therefore, the study through three generations of rats has been conducted. The F₁ generation resulted from mating of animals in which tritium was given only to females, the F₂ and F₃ generation resulted from mating of the parents to both of which tritium was administered.

The effects of tritium on body weight of males and females in F₁ were noticed at the tritium level of 144.3 kBq/g of food, and of 185.0 and 370.0 kBq/ml of drinking water. The decrease of weight in this generation was permanent in the group exposed to tritiated food, while in the tritiated water groups the decrease of body weight existed only up to 35 days of age. In Experiment 1 with low tritium activities the decrease in body weight of exposed animals was observed only in F₂. This decrease continued with some fluctuations throughout their lifetime. The body weight of F₃ neonates was essentially unchanged.

Laskey, et al.,² reported sporadic differences in body weight which occurred randomly with time and dose level in either sex of F₁ rats exposed to tritium in drinking water at the concentrations from 0.48 to 481.0 kBq/ml. In other experiments, Laskey, et al.,³ also found decrease of body weight of 130-day old males of F₁ lifetime exposed to HTO in drinking water at the concentrations of 48.1 and 481.0 kBq/ml. However, no significant reduction in body weight was observed in males during the first 100 days or in females during the whole study.

The above observations lead to the conclusion that chronic tritium exposure can result in stunting of rats at various ages and it is more pronounced in males.

The tritium exposure at low activities had no influence on relative organ weights of F₁. A slight decrease of relative weights of several organs was found in 21-day old females of the F₂ generation in which the dose absorbed in tissues ranged from 6.7 to 8.9 cGy.

The decrease of relative brain weight of F₃ neonates confirms a HTO effect previously observed in F₂ neonates, exposed to similar tritium activity in drinking water.²

The most evident effect of tritium exposure has been noticed in the testis. The effect was manifested in reduction of their relative weight and sperm count. It is known from other studies that the most easily recognized effect of exposure to ionizing radiation is marked change in testicular weight.¹¹ In our experiment the testis weight was already changed in males exposed to HTO in activity of 185.0 kBq/ml. In other studies^{1,3} lifetime exposure to 480.0 kBq/ml of HTO in drinking water caused depression of testis weight and similarly as in our study exposure to HTO in concentrations one order of magnitude lower had no effect on weight of testis. The tritiated food exposure in activities used in Experiments 1 and 2 did not affect the weight of testis.

Unexpected large reduction in sperm count was found in 71 day-old males exposed to tritiated food, where the absorbed dose in testis was as low as 44.0 cGy. Almost the same reduction in sperm count was found in group of males exposed to the highest tritium activity in drinking water, in which the absorbed dose was 162 cGy, i.e., about four times higher (Figure 1). This would suggest that tritium given in organically bound form is much more effective on sperm production than HTO. The concentration of tritium in dry testis tissue after HTO exposure at activity of 370.0 kBq/ml was equal to 53.58 kBq/g, while in dry testis of males exposed to 144.0 kBq/g of tritiated food it was only 29.19 kBq/g. It seems to indicate that total concentration of tritium organically bound in the tissue does not determine the changes in sperm production. Responsible for this might be rather microdistribution of tritium atoms in cells. It is known from other studies^{4,5,6,7} that the biological effectiveness of tritium depends on the tritiated organic compounds entering the organism, e.g., investigation by Rytoemaa, et al.,⁷ has indicated that ³H-leucine, ³H-uridine and ³H-thymidine were for growing cells of chloroleukaemia about 10, 100 and 1,000 times as toxic as HTO, respectively.

Sperm production in testis after HTO exposure is directly related to the absorbed dose in this organ, indicating that damage depends on the amount of energy absorbed in this tissue. Quadratic relation between dose and the sperm production in testis of males exposed to tritiated food seems to indicate the existence of additional factors responsible for greater effectiveness of tritium. They may be connected with incorporation of tritium into radiobiologically more effective sites. However, it should be emphasized that the number of experimental data used for calculation of the equations was not sufficient to make this finding entirely reliable and additional studies are required.

A cumulative effect of exposure on litter size was not observed. In all generations, the litter size in exposed groups was reduced to the same extent. Also no dose-effect relationship was observed in the F₁ generation, in which five groups with various activity level and two different forms of ingested tritium were studied.

Resorptions and ovulations were studied only in the F₃ generation at low level of tritium exposure (Experiment 1). Increase in number of resorptions and preimplantation deaths per litter was noted only in tritiated food group. These results seem to indicate that severe chromosome defects after T-food exposure can exist. However, the high variability makes this effect questionable.

The results obtained in this study indicate that the influence of tritium on growth of rats and ability to sperm production depend not only on absorbed radiation dose but also on the form in which tritium enters the rat.

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[Text] In the present work the parameters of optocouplers have been discussed and the results of measurements of Polish light emitting diodes (LED's) and photodiodes have been shown. The scheme and characteristics of the fast high voltage optical isolator developed by the authors have been presented. The obtained parameters are as follows: isolation voltage over 70 kV, t_d --delay time is less than 50 ns, stability $\frac{\Delta t_d}{\Delta I_{LED}} \leq 0.5 \text{ ns/A}$ with $I_{LED} \geq 5A$.

I. Introduction

Many research systems influence measuring and control instruments because of electromagnetic interference produced during their work.

The interference can be partially eliminated by the application of optoelectronic devices which enable the transmission of signals with no electrical link between measuring instruments and equipment controlling the research system. Typical method which solves this problem is using the optocoupler.¹ Since available optocouplers provided neither enough high insulation voltage or small delay time, it was necessary to design a fast, high voltage optocoupler mainly to convey the synchronization pulses into and from measuring and control instruments.

This paper presents the characteristics of commercially available optocouplers as well as the parameters of the optocoupler designed in the Institute for Nuclear Research. The optocoupler provides following parameters: maximum delay time--100 ns; insulation voltage--70 kV; and output pulse height--100 V.

II. Optocoupler's Characteristics

The typical optocoupler consists of a Light Emitting Diode (LED) optically coupled to either a photodiode either a phototransistor or a photothyristor.²⁻⁴

The basic feature of the optocoupler is the galvanic separation between LED at the input and photodetector at the output. The insulation gap is filled either by the air, either by an epoxy resin, either by a glass or by optical fibers.

The optocouplers may be used in these applications which demand electric insulation between two systems, or small coupling capacitance. In particular they are useful in communication links, measuring and control equipment for high voltage systems, in medicine and health service where the patient must be absolutely insulated.

The optocoupler can be characterized by its delay time, input-output insulation voltage and Current Transfer Ratio (CTR) i.e., the output current to the input current ratio. Optocoupler's characteristics depend upon its construction and technology nevertheless its parameters relate to each other. For example CTR is inversely proportional to the insulation voltage, as they both depend on the gap length. The ten-fold increase of the insulation voltage would diminish CTR by nearly one order of magnitude.

On the whole the switch time of optocoupler depends upon the speed of both, LED and photodetector. The most of LED's provide the switch time of 1 to 10 ns. The phototransistor's switch time is of 1 μ s and photodiode's of 1 ns but it should be emphasized that the decrease of diode switch time causes the decline of its quantum detection efficiency which limits CTR. That is why usually the speed of the photodetector limits the speed of the whole device.

Table 1 compares some characteristics of several commercially available optocouplers.⁴ It shows that optocouplers built of LED and photodiode offer the smallest switch times, while the greatest gain-bandwidth is offered by an integrated combination of photodiode and amplifier at the output.

Table 1. Basic Maximum Characteristics of Various Optocouplers

Optocoupler type		Maximum current transfer ratio percent	Switching time as cut-off frequency MHz	
Input	Output			
E D (GaAs)	phototransistor	150	2	(0.2)
	photodarlington	500	10	(0.03)
	photodiode	0.2	0.02	(15)

Commercially available optocouplers are usually manufactured in typical packages used in microelectronics for which insulation voltage is of approximately 5 kV.

III. Optocoupler's Design

Construction Presumptions

The efforts to design the optocoupler were undertaken by us because none commercially available met our demands: very high speed and high voltage insulation of approximately tens of kilovolets. The optocoupler's block diagram is shown in Figure 1. The optocoupler consists basically of three units: emitter, receiver and coupling medium. In the emitter the electric signal, signal, which after passing through the coupling medium initiates the receiver to generate high voltage pulse. This pulse can be used to set the equipment controlling the work of the research system. The short switch time has been ensured by the appropriate LED-photodiode pair yet the requirement for high insulation voltage has constrained us to take advantage of a glass fiberoptic cable type ZN-2243 of Carl Zeiss Jena, length: 2 m; diameter: 10 mm; angle aperture: 35°.

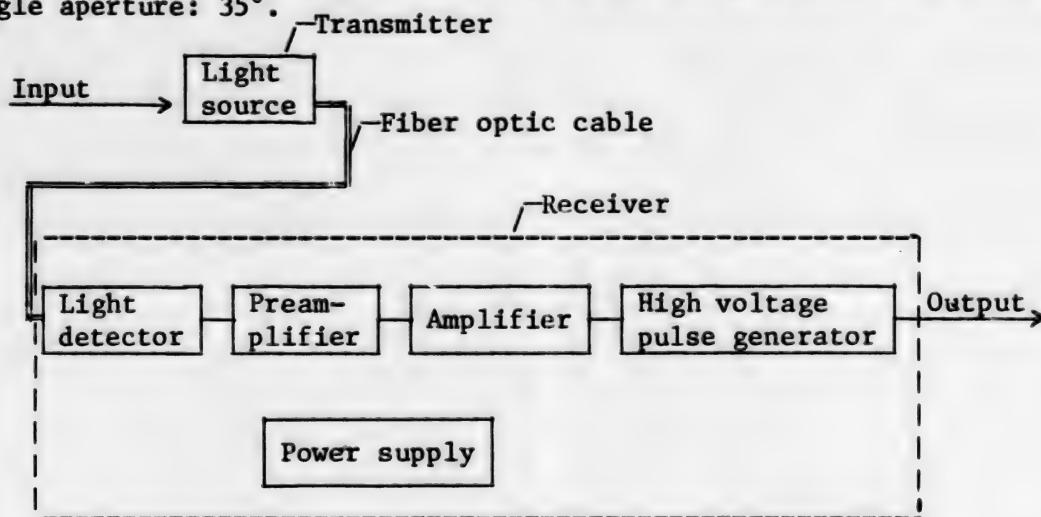


Figure 1. Block Diagram of Optocoupler

1. LED's and Photodiode's Tests

The selection of LED is the most important question prejudging optocoupler's parameters.

In order to match the emitter and detector spectral characteristics a gallium arsenide LED and silicon photodiode were chosen (optimal cooperation in infrared range $\lambda = .9 \mu\text{m}$). Required speed excluded phototransistor since its response time amounts to some microseconds.

Tables 2 and 3 show the basic parameters of Polish LED's⁵ and photodiodes.⁶ Some of them are commercially available and others are under development. Since the parameters were not very precise and the dynamic characteristics were not given we had to test all fast diodes to match them optimally. The tests appear also indispensable to measure CTR of LED-fiber-photodiode set which highly depended upon interstitial energetic losses.

Table 2. Characteristics of LED's Developed in Institute of Electron Technology

Type DEL	Wavelength μm	Maximum forward current mA	Radiation output mW	Cut-off frequency MHz
CQYP-15	0.91	100	0.5	15
CQYP-16	0.95	100	1.5	1.5
CQYP-18	0.91	300	1	15
CQYP-19	0.91	200	1	15
CQYP-20	0.95	200	2.5	0.5
CQYP-25	0.91	500	10	15
CQYP-19A	0.91	200	0.2	15

Table 3. Basic Characteristics of Polish Photodiodes

Type diode	BPYP-30	BPYP-35	BPYP-41	BPYP-44	BPYP-51
Maximum reverse voltage V	100	100	100	100	60
Wavelength μm	0.4-1.1	0.4-1.1	0.4-1.1	0.4-1.1	0.4-1.1
Sensitivity at $\lambda = 0.9 \mu\text{m}$ $U_R=60 \text{ V, A/W}$	0.25	0.25	0.25	0.4 at $U_R=45 \text{ V}$	1 10
Rise time and fall time at $U_R=60 \text{ V, }$ $R=50 \text{ ns}$	12	35	3.5	10	2
	12	35	3.5	10	2

The block diagram of the test circuit is shown in Figure 2. The square wave generator consists of a fast avalanche transistor discharging a long line-coaxial cable (see Figure 3). The generator, driving the LED, delivers pulses of amplitude 50 V, width 80 ns and rise time $\leq 1 \text{ ns}$. Since the photocurrent was of some microamperes and the sensitivity of used oscilloscope was limited the output signal was amplified by the specially designed amplifier. The amplifier provides rise time of 3.5 ns, delay time of 6.5 ns and voltage-current transmittance of 15 kΩ. Its circuit diagram is shown in Figure 4. The amplified pulses of photocurrent were observed on the Tektronix 5444 oscilloscope (rise time 5 ns) simultaneously with pulses driving the LED. Obtained results are shown in Figures 5 to 8.

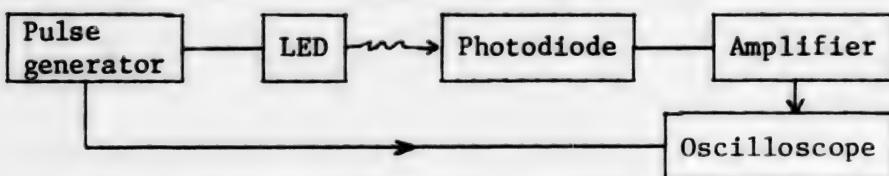


Figure 2. Block Diagram of Test Circuit for LED's and Photodiodes

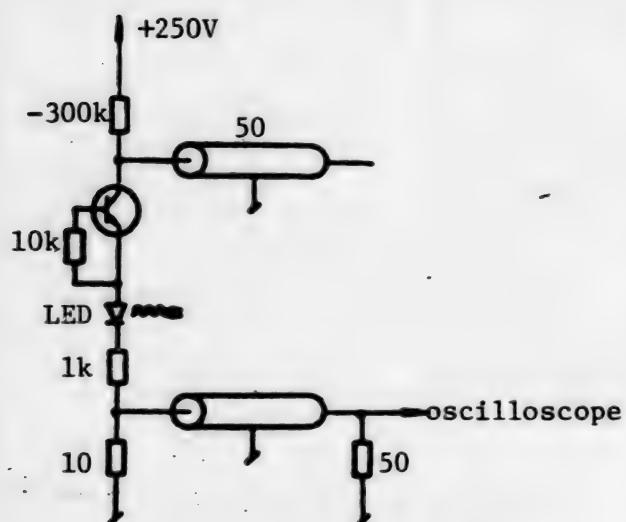


Figure 3. Circuit Diagram of Generator Driving LED

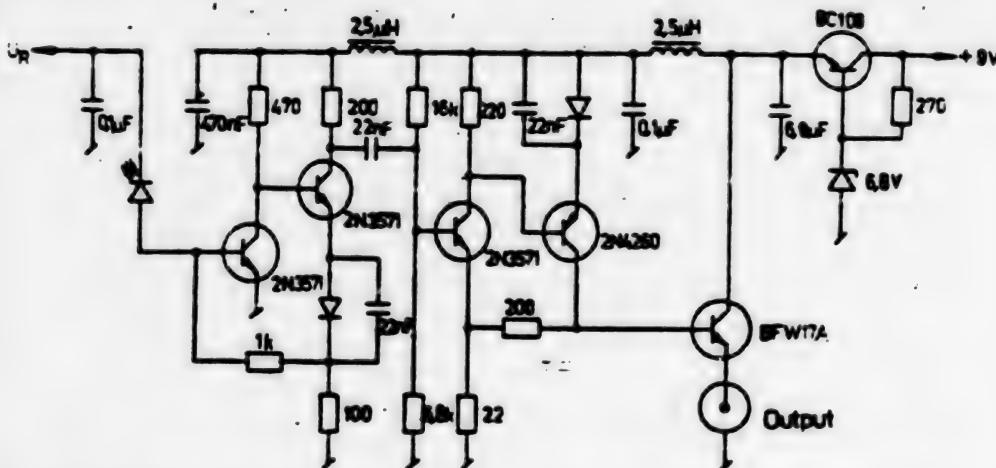


Figure 4. Circuit Diagram of Fast Photocurrent Amplifier

In the LED's testing the fastest of available photodiodes was used as a photo-detector--BPY-77, photocurrent rise time ≤ 0.5 ns according to data sheets. The amplitude and rise time of amplified output pulses were measured as a function of the amplitude of LED forward current. From the amplitude of output pulses and the transmittance of the amplifier the photocurrent was calculated (see Figure 5). All measurements were performed with the same photodiode so its current directly corresponded to the relative efficiency of LED's energy conversion. The rise time of LED's light pulse calculation regarded the rise time of photodiode, amplifier and oscilloscope (see Figure 6).

In the photodiodes' testing, the fastest of available LED, i.e., the CQY-11B of measured rise time $t_r = 6.6$ ns at $I_{LED} > 0.6$ A was used as the source of light. The amplitude and rise time of output pulses were measured as a

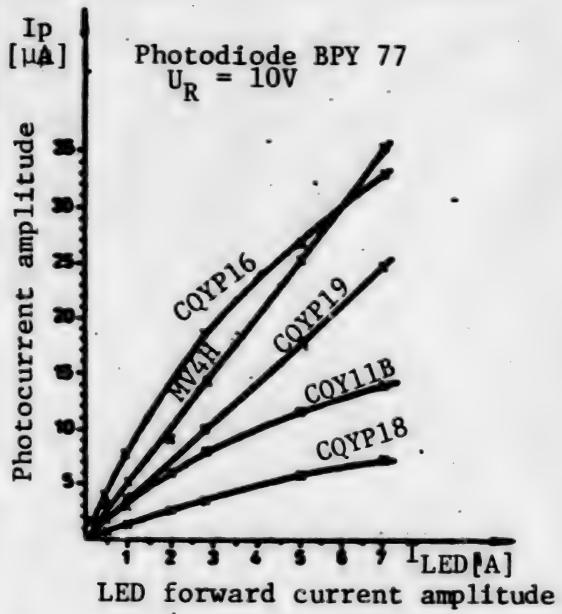


Figure 5. Photocurrent vs. Forward Current for Different LED's

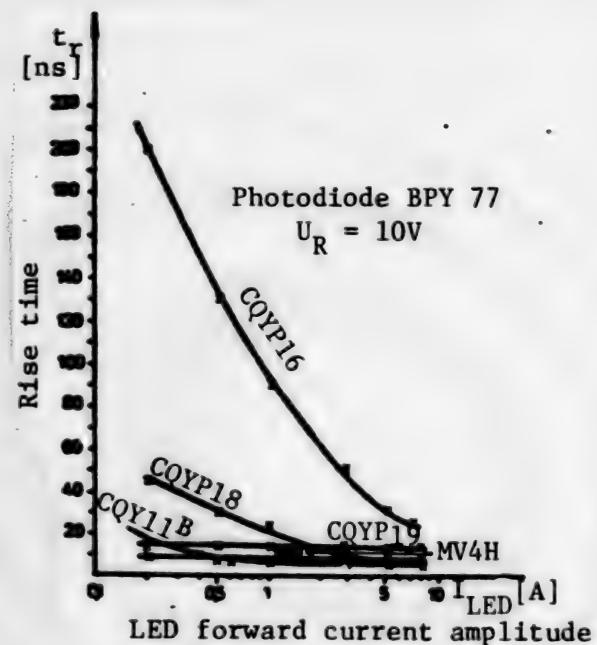


Figure 6. Rise Time of Light Pulse vs. LED Forward Current for Different LED's

function of the bias voltage of the photodiode. Taking into account the amplitude of output pulses corresponding to photocurrent one can compare the LED's light detection efficiency of different photodiodes to one another (see Figure 7). The rise time of the photocurrent was calculated having regarded the rise times of LED, amplifier and oscilloscope (see Figure 8).

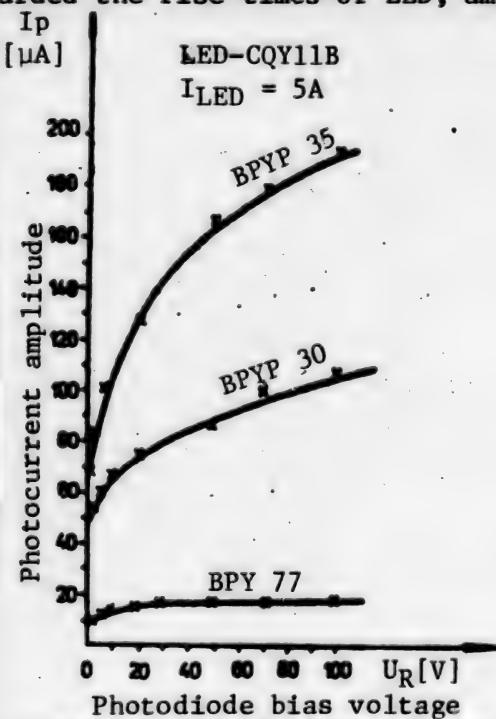


Figure 7. Photodiode Efficiency vs. Bias Voltage

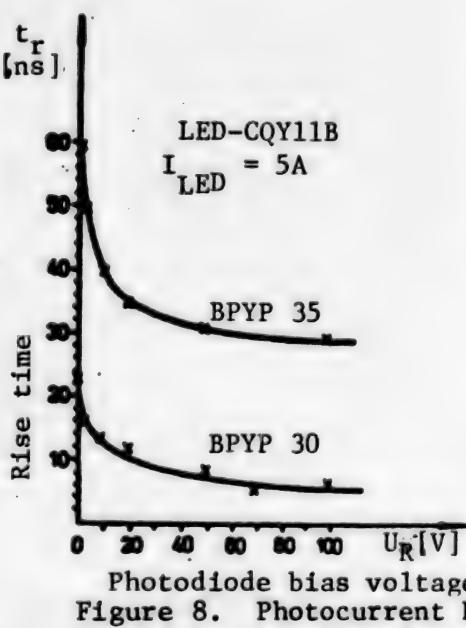


Figure 8. Photocurrent Rise Time vs. Bias Voltage

On the basis of obtained characteristics the LED's and photodiodes could be compared to one another. The best efficiency of energy conversion was discovered in the Polish LED type CQYP-16, but its rise time was also the greatest. Moreover its rise time highly depended upon forward current and this could involve the instability of optocoupler delay time. The smallest rise time among Polish LED's was found in CQYP-19. Despite its energetic efficiency, it is only a bit worse than that of CQYP-16 and of MV4H its rise time of 15 ns is only slightly greater than those of other fast diodes.

The range of LED's current (up to 8 amps) needs the explanation. The tested LED's are low power devices with maximum forward current of hundreds of milliamperes. The forward pulse current, however, could be greater if maximum DC power dissipation was not exceeded. In practice the LED device, when operated under stress conditions, is usually expected to shorten its lifetime and to decrease its energetic efficiency.²

The measured photodiode characteristics show that fast photodiodes' spectral efficiency is lower and that maximum bias voltage enables it to achieve both the shortest rise time and the greatest spectral efficiency.

Two Polish photodiodes have been tested. The faster one, BPYP-30 of rise time $t_r < 10$ ns at $U_r > 50$ V, offers great efficiency-bandwidth product. The value of this product can be accepted to be the diode suitability criterion because it determines the requirement for the receiver's amplifier parameters.

For reasons mentioned above the CQYP-19 and BPYP-30 were used to design the optocoupler.

Figure 9 shows the dependence of the optocoupler's CTR on photobias voltage. CTR is not dependent upon the CQYP-19 forward current (see Figure 5). Obtained value of CTR (10^{-5}) is much less than those of Hewlett-Packard optocouplers (see Table 1) at cut-off frequency of approximately 20 MHz. This could be explained by interstitial losses in the optocoupler and by relatively low energetic efficiencies.

2. Receiver Description

The optocoupler's receiver consists of the photodiode, preamplifier, main amplifier and high voltage pulse generator. The preamplifier, directly driven by photodiode, is of particular importance. Its bandwidth should not be less than the photodiode's, and it should provide high sensitivity. The output resistance of severely biased photodiode is high, therefore it can be treated as a current source. There are two possible input stages: 1) the photodiode in series with a resistor driving a voltage amplifier of high input resistance, greater than the value of the series resistor; and (2) the photodiode drives directly a current-voltage converter (current amplifier). The second version provides greater bandwidth determined among others by the input circuit time integration factor whose value is relatively small with regard to small input resistance amounting to approximately tens of ohms.

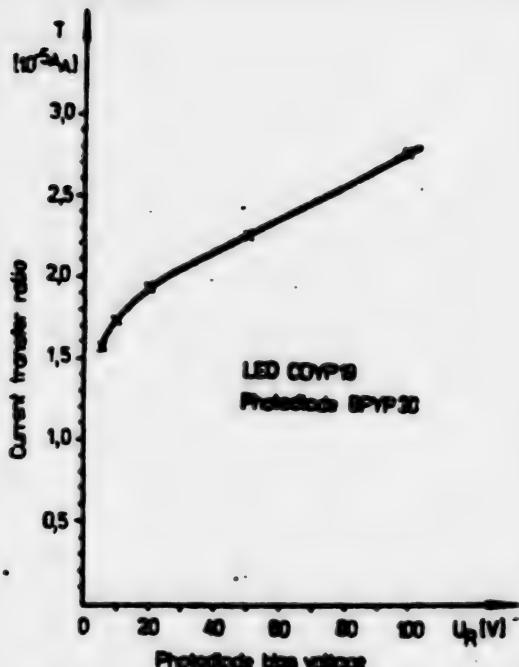


Figure 9. CTR vs Bias Voltage

The scheme of the proposed amplifier is shown in Figure 10. It is based on two high frequency transistors T_1 and T_2 (BF-200) with negative feedback proved by the resistor R_4 . The current gain of the stage amounts to 60 A/A, the current-voltage transmittance to approximately 40 k Ω and rise time is less than 10 ns. The photodiode is biased by 70 V and drives the converters input directly.

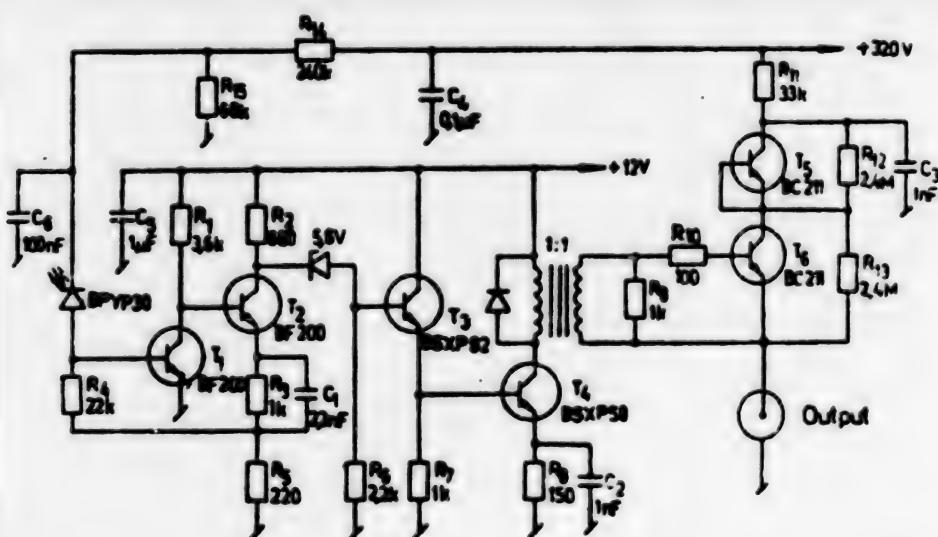


Figure 10. Circuit Diagram of Optocoupler Receiver

Specification of the amplifier's parameters should be preceded by the determination of the threshold current value of LED, which would trigger the generator, as well as the generator threshold voltage.

The LED forward current must compromise two limitations: 1) very low current complicates the output amplifier construction; 2) the high one limits the pulse repetition for the LED's average current must be kept below its maximum value.

Therefore the threshold current value of the LED should be less than the maximum average current of the CQYP-19, i.e., 300 mA.

The generator threshold voltage depends on its network solution. Presumed output pulse, $U_j > 100$ V, and the rise time $t_r \leq 5$ ns at 50Ω load can be attained in the simplest way by a circuit with two avalanche transistors in series.⁷ The disadvantage of this solution is that the interstage transformer to drive the amplifier must be applied (see T5 and T6 in Figure 10). This circuit is triggered by the amplitude of approximately 1 V.

Taking into account LED minimum current, CTR of the optocoupler, voltage gain of the preamplifier and the generator threshold voltage, we estimate the voltage gain of the amplifier at 5 V/V. The proposed circuit consists of the emitter loaded amplifier T3 driving the transformer amplifier T4 with emitter correction network to raise the gain in the high frequency range. The measured parameters are: voltage gain: 12 V/V; rise time of approximately 25 ns at the load of 1 kΩ.

IV. Optocoupler's Model Testing

Testing was aimed mainly to verify the usefulness of the optocoupler in fast synchronization equipments which require small delay time and its high stability.

The delay time t_d measured between the input pulse of the LED and the output pulse of the receiver is shown in Figure 11. The delay time amounts to 96 ns at $I_{LED} = 0.3$ A and decreases to 42 ns at 10 A. This could be explained by two effects: the trigger delay time of avalanche transistor depends on the amplitude of triggering pulse and on its rise time. At $I_{LED} = 10$ A the trigger delay time of the generator amounts to 12 ns. The other optocoupler's stages delay times are the following: the LED and photodiode: 15 ns; the fiber 10 ns; the preamplifier: 5 ns. Stability of the optocoupler's delay time depends mainly upon the stability of LED forward current. The influence of temperature is negligibly small.⁸ At forward current $I_{LED} \geq 5$ A the stability fulfills the following inequality: $\frac{\Delta t_d}{\Delta I_{LED}} \leq 0.5$ ns/A.

The maximum repetition rate of the optocoupler amounts to 10 kHz and is limited by the avalanche transistors.

The parameters of output pulse are the following: amplitude $U_i = 150$ V, rise time $t_r = 5$ ns, pulse duration: 50 ns, at the load of 50Ω (see Figure 12).

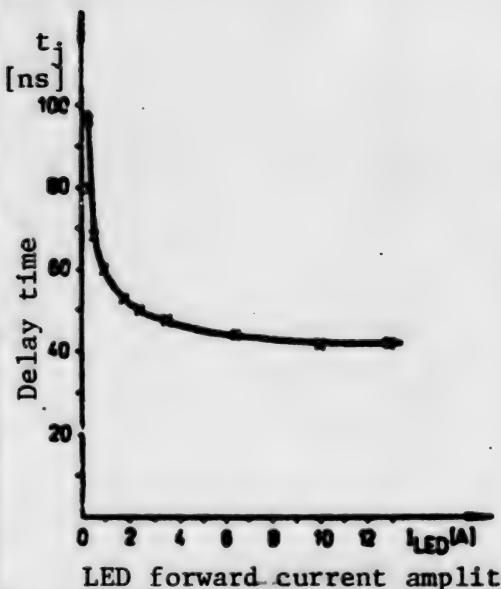


Figure 11. Optocoupler's Delay Time vs LED Forward Current

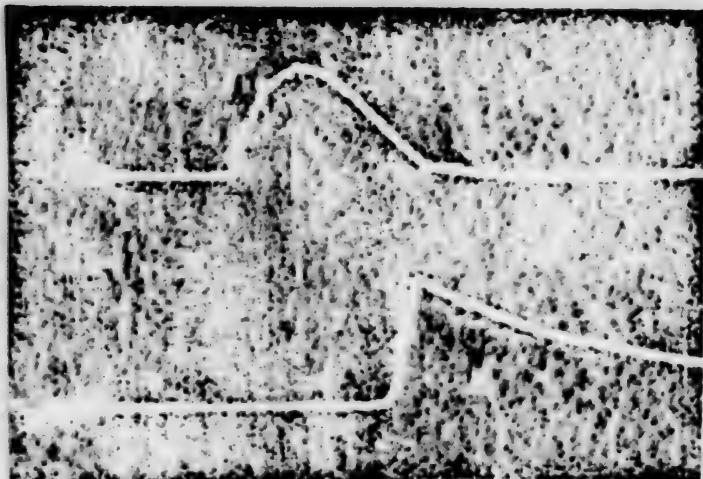


Figure 12. Oscillogram of Photo-current (upper trace, 5A) Div, 20 ns (div) and of Output Pulse of Optocoupler (lower trace, 100 V/div)

The amplitude and the rise time of input pulse depend upon the parameters of the avalanche transistors, while the pulse duration could be increased by the increase of the capacitance C3. The avalanche transistors were experimentally selected out among Polish low-frequency commercial transistors.

The voltage strength tests were performed mainly with the fiber. Up to 70 kV there were no breakdown effects.

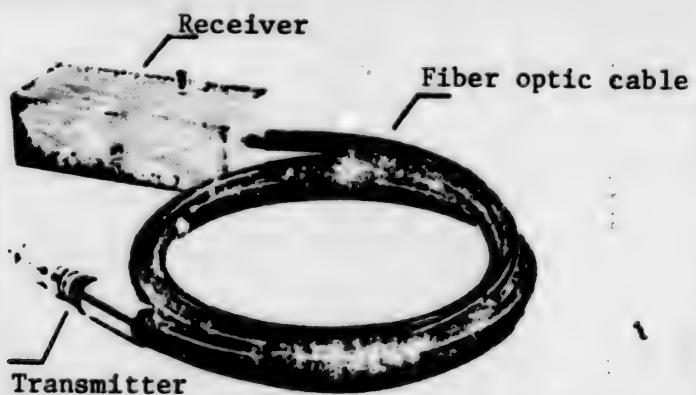


Figure 13. Optocoupler

V. Conclusions

On the basis of measurements it may be claimed that designed optocoupler may be used to transmit synchronization signals of measuring and controlling instruments of research systems.

Moreover, it may be useful for transmission of both analog and digital signals up to 15 MHz without the output generator. The measurement's results have shown that optocoupler's linear distortion is less than 2 percent within two-decade range of LED forward current.

The measured parameters of the optocoupler i.e. the delay time $t_d \leq 42$ ns, at $I_{LED} \geq 10$ A, stability $\frac{\Delta t_d}{\Delta I_{LED}} \leq 0.5$ ns/A at $I_{LED} \geq 5$ A could be considerably better if new semiconductor optoelectronics devices were in use.

In optocouplers designed only for transmission of synchronization signals the LED could be replaced by a semiconductor laser, and the photodiode by an avalanche one. Semiconductor lasers provide higher efficiency of coupling with fibers (20 to 80 percent), smaller rise time of approximately some nanoseconds and higher quantum efficiency of up to 30 percent in comparison with 1-5 percent of LED's. The avalanche photodiode enables it to achieve photo-current rise time less than 2 ns at the efficiency of 1 to 10 A/W, ten times greater than p-i-n as p-n diodes do (see Table 3). The optocoupler assembled of the laser, fiber and avalanche photodiode can provide CTR approximately 100 times greater, and delay time by approximately 10 times shorter than designed optocoupler does. In such devices the problem of fast-rising current generator of 50 A amplitude necessary to drive the semiconductor laser will have appeared. The generator could be designed by using two avalanche transistors in parallel.⁹

Recently the special construction of semiconductor lasers as well as p-i-n and avalanche photodiode has been developed, particularly adapted to quartz single bundle fiber of 0.03 to 0.1 mm in diameter and of suppression coefficient of approximately 10 dB/km. They allow to design optocouplers (in telecommunication called optical links) of cut-off frequency greater than 1 GHz and total energetic efficiency of 10 to 30 percent, which could transmit both analog and digital signals.¹⁰⁻¹³

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Fuel Rod Burnup Rates

Warsaw NUKLEONIKA in English Vol 27 No 9-10, 1982 (signed to press Apr 85)
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[Article by W. Smulek, M. Borkowski and K. Goroncek, Department of Nuclear Chemistry, Institute of Nuclear Research, 03-195 Warsaw, Poland: "Determination of Longitudinal Burnup in an Experimental UO₂-Mg Fuel Rod"; paper received December 1981, accepted February 1982]

[Text] In this work results of the longitudinal burnup determination in a UO₂-Mg fuel rod are presented using radiochemical and mass spectrometric analysis. In the six fuel samples burnup was determined basing on ¹³⁷Cs, ¹⁴⁸Nd and on changes in the isotopic composition of uranium, and a good agreement was found between them. Parallelly, the ratio of radioactivity of europium isotopes ¹⁵⁴Eu/¹⁵⁵Eu was determined as a function of burnup.

Introduction

Burnup of nuclear fuel is, from an economical standpoint, a measure of the fuel utilization. Furthermore, from a technological point of view, the burnup stands as a reference for numerous phenomena which govern the behavior of fuel elements under neutron irradiation. Thus, the knowledge of burnup is essential for fuel development programmes, proper management of power reactors and for nuclear material accounting purposes. The burnup determination can be based on the following approaches:

--theoretical calculations depending on the fuel and reactor parameters,
--nondestructive methods, using γ -scanning or metal (e.g., cobalt, silver) monitors,
--destructive analysis, using internal monitors-components of the irradiated fuel.

In this work attention is given to the destructive methods, i.e. to the methods applied to dissolved fuel samples. Such an approach generally involves:

--determination of selected, radioactive or stable fission products,
--determination of heavy elements (mass > 232),
--determination of isotopic ratios of selected fission products and heavy elements.

Basic Relations

Burnup is commonly expressed as "atom percent fission," i.e. the number of fission which have occurred per hundred atoms of heavy elements initially present in a fuel (fima*). The total atom percent fission (F_t) can be obtained in the following ways^{1,2,3}:

--according to the concentration of a fission product in the irradiated fuel:

$$F_t \text{ (percent fima)} = \frac{N/Y}{N/Y + N_h} \cdot 100$$

where N -- concentration of a selected fission monitor,
 Y -- corresponding fission yield,
 N_h -- concentration of heavy elements,

--on the basis of fractional burnup values:

$$F_t \text{ (percent fima)} = \sum_i F_i$$

where F_i -- atom percent fission of individual fissile isotopes initially present and generated during fuel exposure, calculated from changes in heavy element isotopic abundances,

—indirectly, making use of calibrated relationships involving isotopic correlations of heavy elements or fission products:

$$R_{i/j} = \rho(F_t)$$

where $R_{i/j}$ -- ratio of suitable nuclides.

*Fissions per Initial Metal Atoms

The burnup can be determined most accurately either radiochemically or by mass-spectrometric analysis.

Experimental

Since several years the department is dealing with the radiochemistry of fission products and with destructive methods for the determination of nuclear fuel burnup. These activities aimed at developing selective procedures for the separation and determination of different fission products, including burnup monitors, from irradiated reactor fuels. The following separation techniques were applied:

--extraction with the use of different extractants,

--extraction chromatography, using mostly di(2-ethylhexyl) phosphoric acid (HDEHP) and tri-n-butylphosphate (TBP) as extractants,

--ion exchange with Dowex resins.

Extraction chromatography is particularly suited for the separation in question, because of its high effectiveness for small fuel samples. Selective separation schemes have been developed for the fission products listed in Table 1: the corresponding procedures and results are described in.^{4,5,6}

The determination of radioactive burnup monitors was carried out radiometrically. The concentration of stable fission products was measured by means of a mass-spectrometer, type MI-1309 (a single focusing instrument with a multiple filament ion source) using the isotopic dilution technique. The uranium content of fuel samples was determined either potentiometrically or mass-spectrometrically by isotopic dilution using a ²³³U tracer.⁷

The developed analytical methods were applied for the postirradiation examination of UO₂-Mg reactor fuel characterized by:

--uranium content	72.3 percent
--initial enrichment	10 percent in ²³⁵ U
--Mg-content	13 percent
--cladding material	Al
--irradiation period	126d
--cooling time	12-19y
--neutron flux	$10^{13} n/cm^2 \cdot sec$

Six samples were investigated and withdrawn from an experimental fuel rod. The fuel samples were treated with 6 M HNO₃; under these conditions only the UO₂-Mg, but not the cladding material, dissolved. After proper dilution, part of the obtained stock-solution was evaporated to dryness, again dissolved in 0.1 M HNO₃, and divided into four aliquots. Two aliquots were separately marked with ²³³U and ¹⁵⁰Nd tracers. These operations were carried out quantitatively by weighing. The diagram for the separation of uranium, neodymium, caesium, cerium and europium is presented in Figure 1.

Table 1.

Nuclide	Half-life	Fission yield (percent)			Gamma energy measured (keV)	Relative intensity (percent)
		^{233}U	^{235}U	^{239}U		
^{95}Zr	65 d	6.1	6.4	5.0	724 758	43 54
$^{95}\text{Nb}^{\text{a}}$	35 d				768	100
^{103}Ru	39.7 d	1.6	2.9	5.6	498	90
^{106}Ru	1.0 y	0.22	0.38	4.57		
$^{106}\text{Rh}^{\text{a}}$	30 a				513 624	21 11
^{137}Cs	30 y	6.5	6.15	6.5	-	-
$^{137m}\text{Ba}^{\text{a}}$	2.6 min				662 162	84.6 5.0
^{140}Ba	12.8 d	6.7	6.4	5.6	537	25
$^{140}\text{La}^{\text{a}}$	40.2 h				1600	95
^{144}Ce	285 d	4.8	5.6	3.8	133	5.9
$^{144}\text{Pr}^{\text{a}}$	17.5 min				696	1.2
^{148}Nd	stable	1.4	1.7	1.7	-	-
$^{153}\text{Eu}^{\text{b}}$	stable	0.11	0.14	0.34	87	32
^{155}Eu	1.8 y	0.010	0.03	0.16	105	20.2

^a Isotopes formed by disintegration of the mother^b Target nucleus for $^{153}\text{Eu}(n,\gamma) ^{154}\text{Eu}$

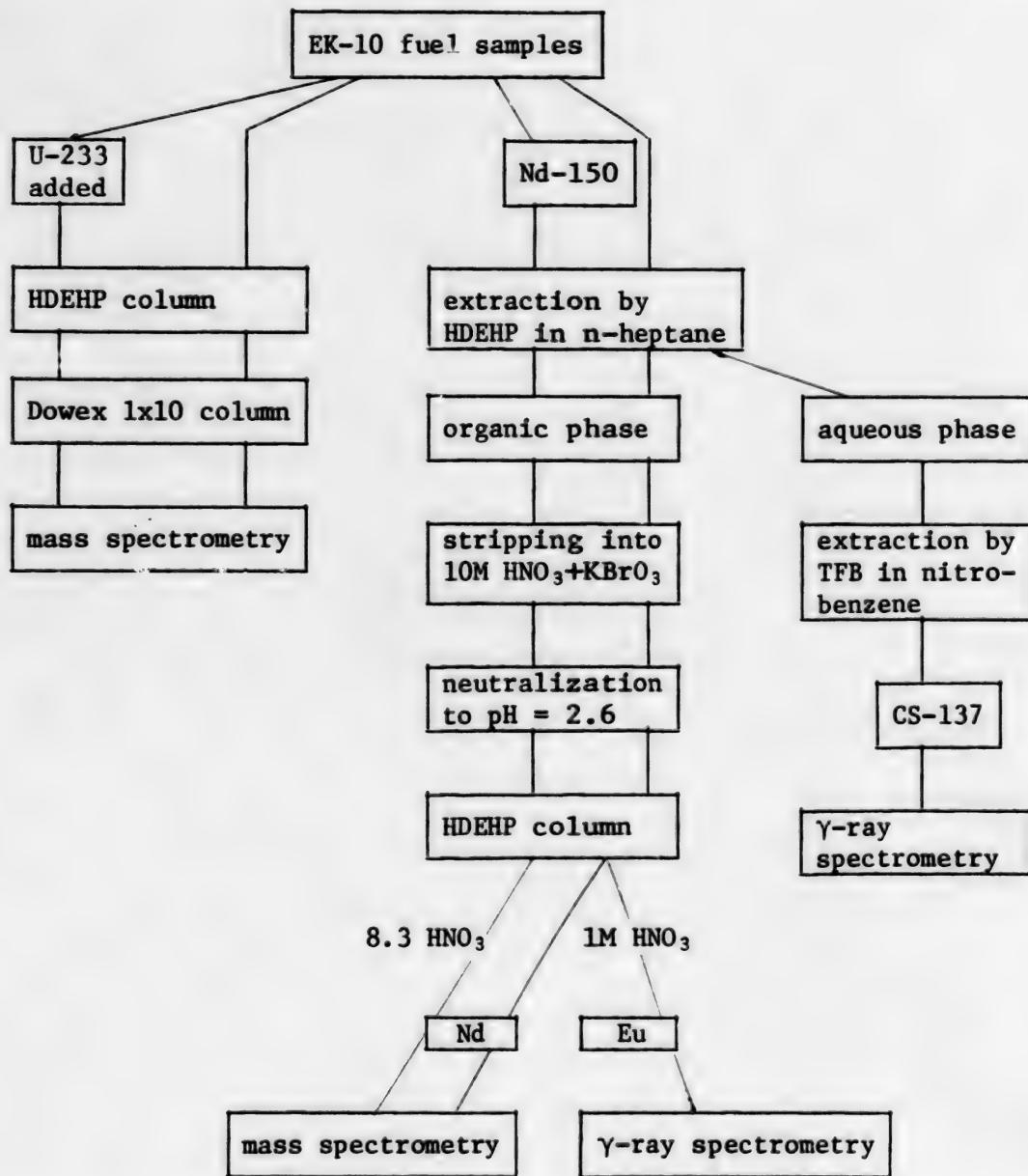


Figure 1. Separation Scheme for Uranium, Caesium, Neodymium and Europium

Result and Discussion

Beside investigation on individual burnup monitors like ^{148}Nd , ^{137}Cs , ^{144}Ce , experiments were also carried out on the application of fission product ratios to the burnup determination. A promising ratio is formed by the radioactive isotopes ^{154}Eu and ^{155}Eu , which have a long half-life (16y and 5.y respectively) and a different formation reactions, the main routes being $^{153}\text{Eu}(n,\gamma) ^{154}\text{Eu}$ and $^{155}\text{Sm} \xrightarrow[\text{23.5min}]{\beta^-} ^{155}\text{Eu}$. The main advantage of using such an isotopic pair is that the separation yield can be neglected and the

absolute determination of radioactivity is not necessary. Figure 2 shows the elution curve of individual fission products and reveals the selectivity of extraction chromatography. A gamma-spectrum of the europium separated from the irradiated fuel is shown in Figure 3. The summarized burnup values for the fuel rod are presented in Figure 4. The ratio of radioactivity of $^{154}\text{Eu}/^{155}\text{Eu}$ is proportional to the burnup values determined and proved to be a useful and convenient indirect monitor. The longitudinal burnup profiles of the fuel rod, determined according to ^{137}Cs , ^{148}Nd monitors and changes in the uranium isotopic composition, indicated a similar distribution. The differences between the Cs- and Nd-method were within 2-5 percent, thus the accuracy of burnup determination could be considered as satisfactory. The more distinct differences at the rod ends may be due to caesium migration. With respect to the low irradiation level, the contribution of plutonium to the total burnup values was assumed to be insignificant and not taken into account. The ^{144}Ce monitor could not be applied successfully because of the almost decayed activity.

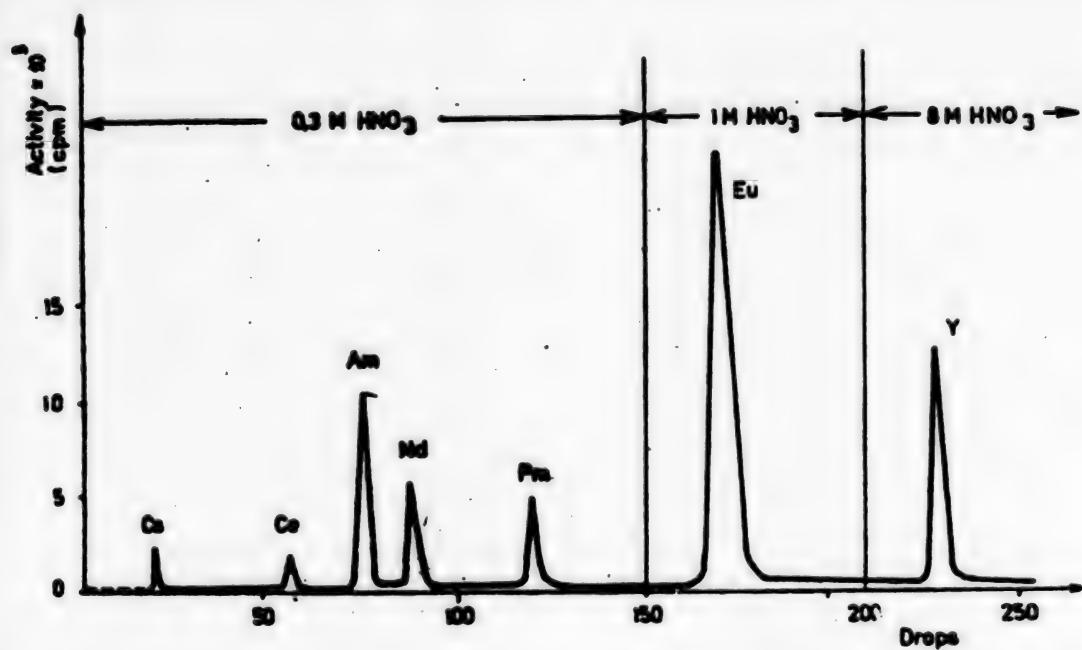


Figure 2. Separation of Some Fission Products and Americium

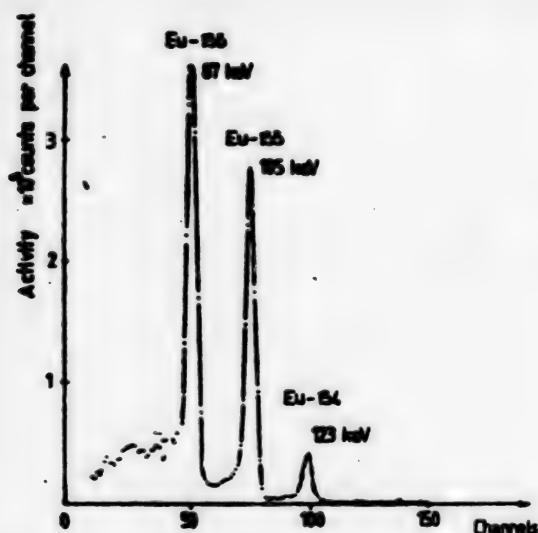


Figure 3. Gamma-spectrum of the Separated Europium

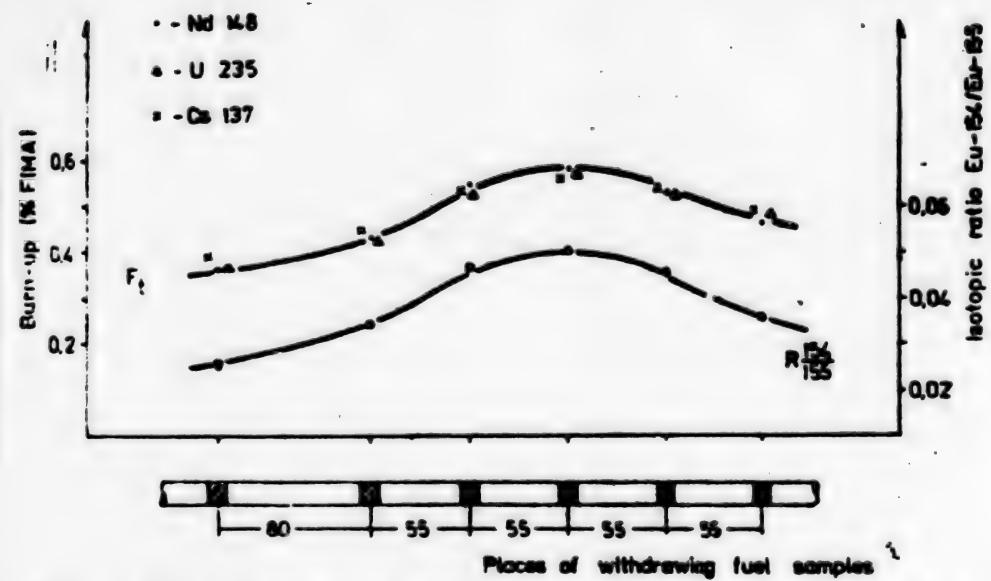


Figure 4. Longitudinal Burnup Distribution in the Fuel Rod

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Radiation-Induced Cell Damage

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pp 447-466

[Article by G.J. Koteles, Frederic Joliot-Curie National Research Institute for Radiobiology and Radiohygiene, Budapest, H-1775, Hungary: "Radiation Effects on Cell Membranes"; received April 1982, accepted July 1982]

[Text] The recent developments in the field of membrane biology of eukaryotic cells result in revival of relevant radiobiological studies. The spatial relations and chemical nature of membrane components provide rather sensitive targets. Experimental data are presented concerning the effects of relatively low doses of x-irradiation and low concentration of tritiated water (HTO) on various receptor functions--concanavalin A, cationized ferritin, poliovirus of plasma membranes of animal and human cells which point to early and temporary disturbances of the composite structures and functions of membranes. References are given to the multifold roles of radiation-induced membrane phenomena on the development and regeneration of radiation injuries.

Introduction

Since the earliest days of radiation biology the radiation effects on cell membranes were always noted. The depth of knowledge, of course, was dependent on the actual level of cell biology. In the last decade the new discoveries and theories concerning the structure and function of cell membranes like the fluid mosaic model¹ put the relevant radiobiological studies into new perspectives. The role of membrane-system as one of the possible targets contributing to the development of radiation effects on cells has to be considered again and the new concepts as well as the experimental data may also have some influence on the theory and practice of radiation protection.² In the present paper those aspects like permeability features, radiation chemistry of membrane components, etc. covered by excellent reviews³⁻⁵ are not mentioned except a few

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areas which point to radiosensitivity of the complex structure and function of plasma membrane, examples are given for the functional alterations of lectin-binding sites and virus-receptors and finally, some possible relationships are raised between the membrane alterations and various cell functions.

On the Scenery of Events

The micromorphological observations of any cells and cellular organelles clearly suggest that the system including all types of membranes--i.e., plasma membrane, intracellular network of Golgi-endoplasmic reticulum, external and internal membranes of mitochondria, the microtubuli, etc.--represent a considerable volume for energy absorption. For example, the endoplasmic reticulum occupies approximately 15 percent of the total cell volume in liver, which is about 2-3 times larger than the nucleus.⁶ The basic spatial arrangements of chemical components, the lipid bilayer, the integrated or transmembrane proteins form a membrane structure of approximately 10-15 nm thick with an additional 8-10 nm external layer consisting of the extruding carbohydrate and oligopeptide side chains of glycoproteins. These volumes have to be considered for microdosimetric assessments or modeling. It is interesting to note that these dimensions are comparable with those applied for elementary target models of nuclear DNA--a 3.4 x 2 nm cylinder which corresponds to one complete turn of the double helix.⁷ The intermolecular contacts of lipids (30-50 percent: neutral fats, phospho- and glycolipids and 50-60 percent proteins) are insured by rather weak hydrophobic bonds. The energy barrier against pulling a glycophorin protein molecule through the red blood cell membrane amounts to approximately 60 kcal/mole-- 2.6 eV per molecule which is about the same energy as that of a single covalent bond and it is interesting to note that one-tenth of this energy holds a ligand to a receptor.⁸ The lipids and proteins form very complex functional units, sometimes called "signal clusters" in case of receptors, because the integrated proteins may act as enzymes on the surrounding phospholipids and the product assists for the signal-transmissions as revealed recently in the case of methyltransferases and methylation of phosphatidylethanolamine to phosphatidyl-choline.⁹ The phospholipids and their ratio to cholesterol determine the local densities and fluidities in certain domains of membranes, the alteration of which--the phase transition--is cooperative, i.e., the greater mobility of a fatty acid induces that of the neighboring one. By this way the process can amplify small perturbations of a membrane region and form and pass signals.¹⁰ On the contrary, due to their relatively rigid structure, transmembrane proteins inhibit the movement of fatty acids in their environment and form less fluid domains. It is quite probable that each type of protein or enzyme has an optimal concentration and composition of phospholipids around itself. Therefore, the enzyme activities of integrated proteins might be regulated and influenced by the surrounding lipids. The stimulation of lymphocyte plasma membrane by concanavalin A induces a rapid change in the fatty acid composition of membrane with the accumulation of a polyunsaturated fatty acid, arachidonic acid (20:4).¹¹ It has also been demonstrated that the activity of certain membrane bound enzymes in lymphocytes (like the Na^+/K^+ -dependent ATPase and lysolecithin-acyltransferase) are also influenced by the surrounding concentrations of various phospholipids.¹²⁻¹⁴ From the examples presented it is quite obvious that the spatial and intermolecular arrangements

of membrane components provide dynamic and easily reactable entities which mediate innumerable cellular processes under physiological conditions and these can be disturbed even by mild action of physical, chemical or biochemical agents like ionizing radiations, drugs, metabolites.

Lipoxygenation

One of the basic mechanisms which result in the rapid disturbance of membrane structures and functions is the lipoxygenation. The membrane lipids maintaining such important structural and functional tasks as those listed above seem to be the most radiosensitive compounds among the biomolecules. The finding that the number of affected molecules per 100 eV energy absorption, the G-value, is much higher in lipids than in proteins and nucleic acids^{15,16} points to initiation of a chain reaction in which the affected molecule or the formed organic radical injure other molecules. Thereby the effect is amplified. The chain reaction of lipoxygenation is initiated and sustained by various free radicals including the most reactive ones, the hydroxyl (OH^{\cdot}) and superoxide (O_2^-) radicals and the formation and reactions of various degradation products and modified molecules¹⁷: Both OH^{\cdot} and O_2^- radicals play a role in initiation of lipid peroxidation in human erythrocytes^{18,19} while O_2^- was mainly found responsible for lipid peroxidation of liver, brain and muscle microsome membranes.²⁰ Among the participating substances the polyunsaturated fatty acids (PUFA's) have a central role, since during the process of lipoxygenation they degrade into low molecular weight products of malondialdehyde type. The relative radiosensitivities of fatty acids depend on the degree of unsaturation--the arachidonic acid (20:4) and decosanexaenoic acid (22:6) being the most sensitive ones--and their molecular environments in membranes.²¹ The ease of fatty acid oxidation at pH 7.5 was arachidonic (20:4) > linolenic (18:3) > linoleic (18:2) acid.²² Due to the lessening of PUFA's the phospholipid: cholesterol ratio decreases with the consequence of increased rigidity of membranes and conformational transitions of membrane proteins.²³ PUFA's contribute to the radiation damage of other cell structures by releasing the degradation products into the cell's own nucleus or into other cells and hence, they may lead to the damage of DNA.^{24,25} The chain reaction character of lipoxygenation of membrane constituents is supported by the findings which demonstrate inverse dose-rate effects in liver²⁶⁻²⁸ and in human lymphocytes,^{29,30} i.e., the development of more intensive effects when irradiation is performed at a lower dose-rate than at a higher one.

Elimination of Free Radicals

Free radicals that initiate the chain reaction of lipoxygenation are formed in all cell types during biological oxidation and in such normal cell functions like phagocytosis.^{31,33} Water soluble substances like the NADH-NAD⁺ and vitamin C serve as first line of protection of PUFA's and lipid-soluble substances like vitamin E (the alphatocopherol) play important roles as the second line.³⁴ The radioprotective effect of vitamin E has been known since years,³⁵ and on a molar basis it proved to be the most powerful protector of membrane structure.^{36,37} The alphatocopherol in the normal cellular metabolism is a modulator of lipoxygenation of arachidonic acid and consequently has regulative function in the formation of extra-, and intracellular mediators like prostaglandins, prostacyclins (PGI₂) and various hydroxyeicosatetraenoic acids,

HETE's.³⁸ The membrane stabilizing effect of ubiquinone Q₉ and vitamin K on mitochondria was also reported.³⁹ Among the radicals formed the role of O₂⁻ merits special mention as its formation also runs as a chain reaction involving organic radicals under aerobic conditions.⁴⁰ Its elimination occurs through the superoxide dismutase (SOD) enzymes (superoxide; superoxide oxidoreductase, E.C.1.15.1.1). The balance of these two conditions, i.e. the radical content and the activities of eliminating enzymes determine the actual radical concentration. The possibility of long-lasting presence of O₂⁻ in cells (up to one hour after irradiation) was suggested in case of red blood cells when the addition of a SOD inhibitor, diethyldithiocarbamate was varied in time.⁴⁰ As superoxide radicals are formed in the cellular oxidative processes during normal and other nonradiation induced pathological conditions,³¹⁻³³ SOD enzymes are normally found in cells. The activities of SOD enzymes, however, are different in various cells (Table 1). This condition itself might contribute to the differing radiosensitivities of cells when excess concentration of radicals are formed and maintained through processes related to membrane constituents. Exogenous SOD actually proved to be radioprotective in lymphocytes and model membranes.^{47,48} Endogenously increased SOD content, however, of pathological erythrocytes (Down syndrome) did not decrease the cell's sensitivity against hemolytic and acetylphenylhydrazine injuries.⁴⁹ On the other hand, SOD activity itself is influenced by ionizing radiation (Table 2). The authors listed in Table 2 have found its definite decrease in more sensitive tissues like bone marrow, brain, thymus, thyroid and slight decrease or no changes in less sensitive ones like liver. Although the problem needs further investigations, SOD enzymes might contribute to maintaining the integrity of membrane structures.

Table 1. SOD Activities in Various Cells

Cell type	Enzyme activity unit per mg protein	References
Rat hepatocyte	6.4	41
bone marrow	2.4	41
platelet	2.1-2.7	42
lymphocyte	1.2	43, 44
polymorphonuclear leucocyte	0.6	43, 44
erythrocyte	0.1*	45
Hamster		
CHO	205	46

For SOD enzyme activity units see References 46 and 51.

*U per mg hemoglobin

Table 2. Alteration of SOD Activities in Irradiated Organisms

Animal	Organ	Dose Gy	Effect	References
Rabbit	liver	7.5 - 10	decrease	50,51
Rat	liver	8	no change	41
	liver	8 - 9	no change	52
	liver	8.5	decrease	53
	bone marrow as tissue	8	decrease	41
	bone marrow surviving cells	8	increased	41
	brain	8 - 9	decrease	52
	erythrocytes	6.5	decrease	45
Mouse	thymus	7.5 - 10	decrease	48
	thyroid	7.5 - 10	decrease	48

Radiation-Induced Alterations of Plasma Membrane

The aforementioned conditions and mechanisms influence the integrity of membranes and participate in the development of their radiation-induced functional alterations. In the case of plasma membranes, among others, the surface charge, the activities of membrane-bound enzymes and the function of various receptors are altered upon irradiation.² These properties are based on increasing complexities of membrane constituents, i.e. the surface charges are ensured by ionizable groups on side chains of proteins and lipids under physiological pH, the enzyme activities depend on the conditions of single protein molecules and their close lipid environment, while the proper receptor functions need the coordinated conditions and motilities of several protein molecules as well as of large domains of lipid areas around the proteins. Beside the examples reviewed earlier² concerning the radiation-induced alterations of receptor functions, recent results are presented from our laboratory⁵⁴⁻⁶⁰ which point to the radiation-induced alterations of protein-binding (concanavalin A and cationized ferritin) capabilities and virus receptor activities of animal and human cells.

Cell Surface Alterations of Human Fibroblast

The reactions of mitogen-binding "receptors" against ionizing radiations were studied by labeling irradiated cells with ³H-concanavalin A (³H-conA). It was found that human WI-38 fibroblasts bound increased amount of lectin within the first 3 hours after irradiation in the range of 0.9-9 Gy (Figure 1). After this period the normal binding conditions were reestablished.⁶¹ This early and temporary alteration of cell surfaces was also reflected in the data from scanning electron microscope (SEM).^{59,60} It was observed that while the unirradiated cell culture was confluent and the cell barriers were hardly distinguishable (Figure 2A), 10-60 minutes after 0.25-2.5 Gy the cell-to-cell contacts were loosened, cell edges appeared as membrane ruffles, numerous lamellipodia and filopodia could be observed (Figure 2B, 2C). Within one hour, however, the cells started to settle back through thin plasma bridges and this process has been completed during the first 24 hours. Five Gy,

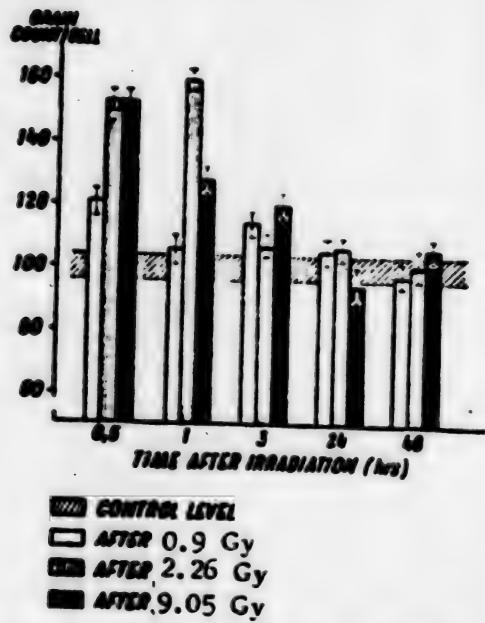


Figure 1. Radiation-Induced Dose- and Time-Dependent Alterations of ^{3}H -concanavalin A Binding by WI-38 Human Fibroblasts. Bars represent S.D.; diagonal hatching, control level; open columns, after 2.26 Gy; solid columns, after 9.05 Gy.

however, gave serious injuries of cells, at the sites of their adhesion only fibronectin-like networks left behind (Figure 2D).

As con A binds to mannose and glucose side-chains of nonsialylated glycoproteins, i.e., which are poor or depleted in neuraminic acid, and consequently in negative surface charges, it was reasonable to check the radiation effects in another way, namely, by cationized ferritin, the binding of which depends on the presence of negative surface charges. The binding could be followed by SEM. Also, a quantitative evaluation could be given as number of particles per μm^2 . Two and a half Gy caused a well-recognizable, approximately 60 percent decrease in the amount of the bound molecules already 10 minutes after irradiation while the intensity of labeling came back closer to the control after 60 minutes. The patchy distribution on the membrane did not change. The data obtained were in good agreement with microelectrophoretic observations.⁶² They indicated a decrease of negative surface charges as cationized protein binding decreased and an increase in con A binding took place possibly due to unmasking of nonsialylated glycoprotein binding sites.

Cell Surface Alterations on Murine and Human Blood Cells

For testing whether the phenomenon, i.e., the early and temporary changes of lectin-binding sites are of limited or general nature, various blood cells of differing radio-sensitivities and of murine as well as of human origins were treated similarly. The bound amounts of ^{3}H -conA could well be detected both with autoradiography and liquid scintillation counting. In the case of *in vivo* irradiated murine lymphocytes the early increase could also be recognized

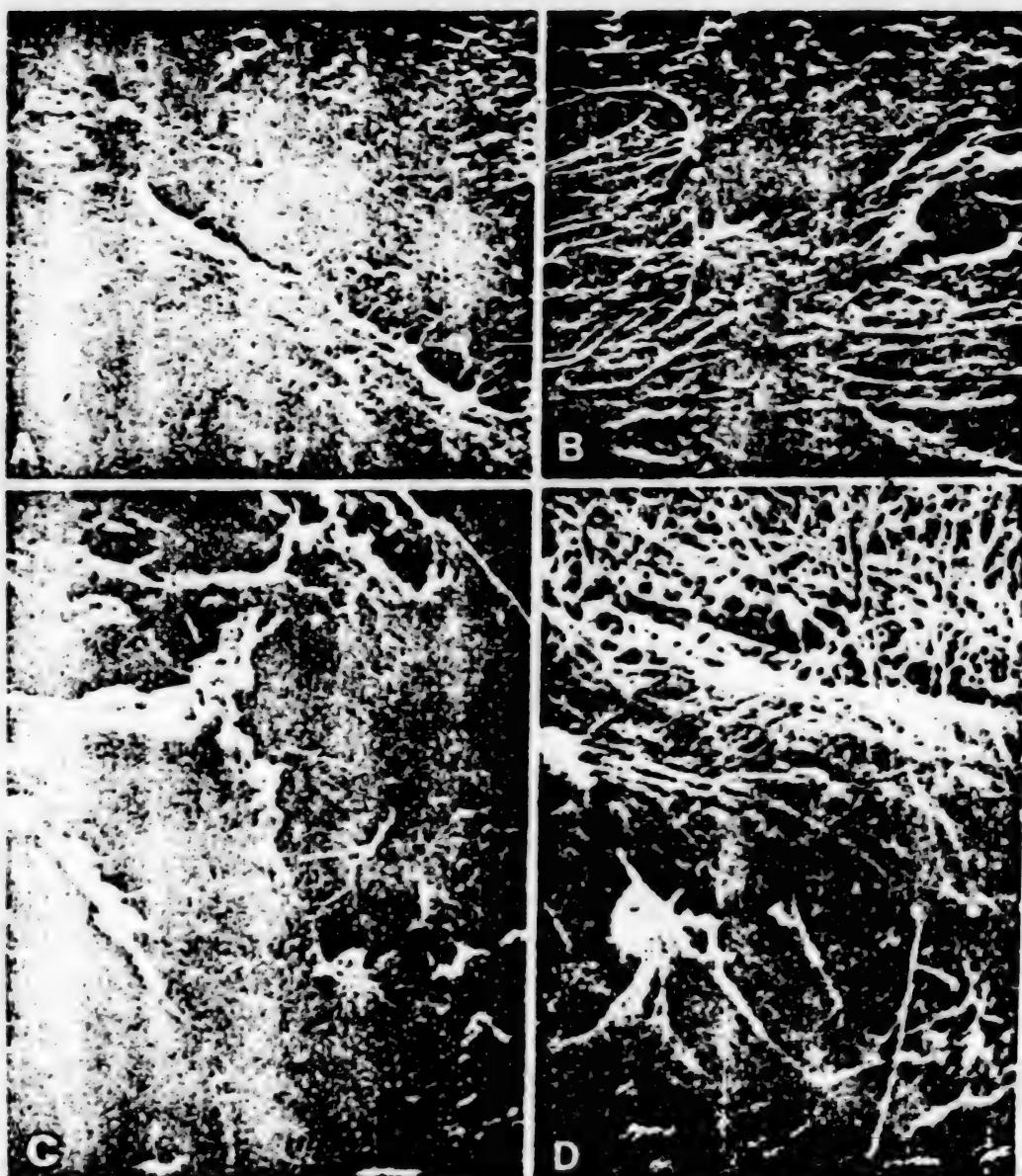


Figure 2. Cell Surface Apparatus of X-Irradiated Primary Human Fibroblasts by Scanning Electron Microscopy

- A: unirradiated control cells, magnification--5,000x.
- B: 1 hour after 0.25 Gy, magnification--4,400x.
- C: 1 hour after 0.5 Gy, magnification--5,000x.
- D: 1 hour after 5 Gy, magnification--6,500x.

after 0.45 and 0.9 Gy, while 4.5 Gy caused only a definite loss of binding down to the level of the specific inhibition by alpha-methyl-D-glucoside (Figure 3). By 24 hours a recovery of lectin-binding was observed in the circulating lymphocytes. The platelets reacted by a multifold increase of con A binding in the first 2 hours between 0.5 and 5 Gy (Figure 4). In human blood cells

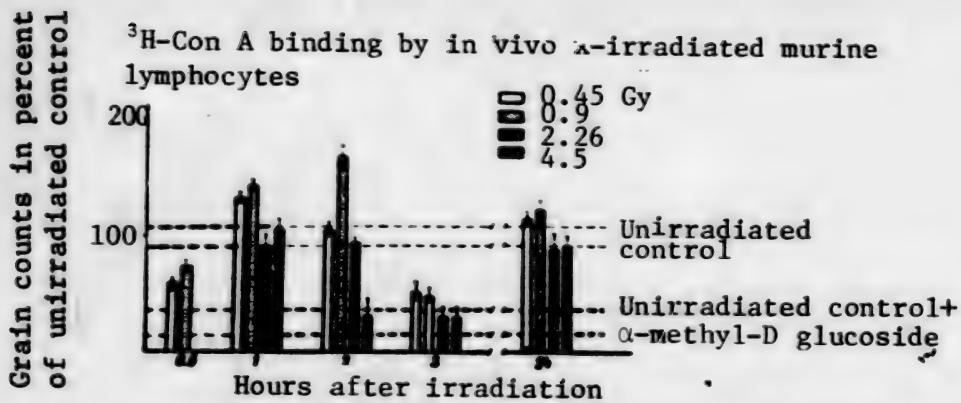


Figure 3. H-Concanavalin A Binding by Murine Lymphocytes Irradiated in Vivo. Mean values \pm S.D. of control and irradiated samples are indicated.

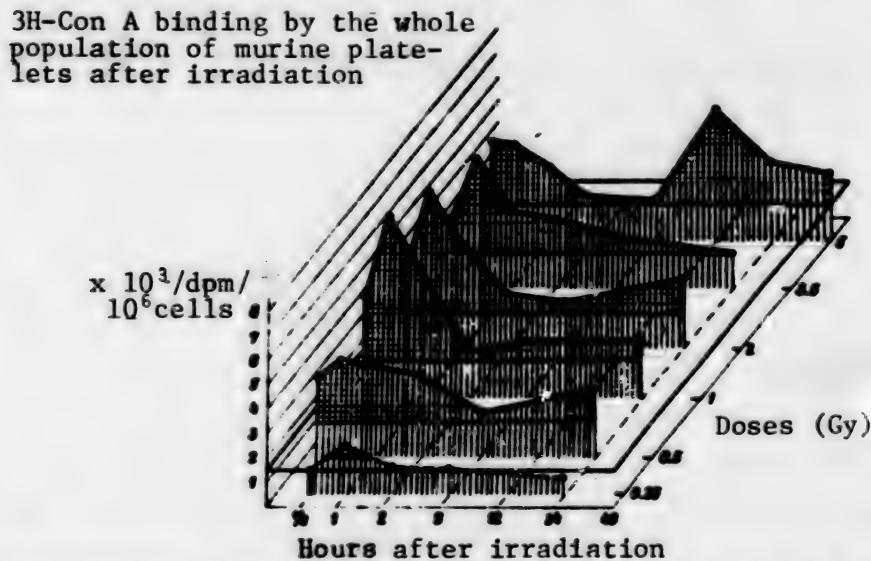


Figure 4. Dose-Dependent Changes in ³H-Concanavalin A Binding to Murine Platelets at Various Post-Irradiation Intervals. The amount of bound lectin expressed in dpm per 10^6 cells is shown on the ordinate.

similar tendencies could be registered after in vitro irradiation (Figure 5). The platelets reacted most sensitively (already after irradiation with 0.1 Gy), then the lymphocytes (0.25 Gy). The least sensitive were the erythrocytes (2-3 Gy). As shown in Figure 5 three dose-ranges can be distinguished according to radiosensitivities, therefore, we suggested the possibility of introducing a biological indicator of radiation injury based on membrane phenomena.⁵⁵ The verification of the applicability of this test after in vivo irradiation is in progress. Besides this dependency of the phenomena on cell types as just mentioned, the effects also proved to be dependent on the physiological conditions of cells, like age, within the cell type. Both in the cases of platelets and erythrocytes the younger subpopulations separated on density gradients reacted more sensitively than the older ones (Figures 6 and 7).

**Dose-dependent changes of con A receptors
of various human blood cells (3 hours after x-irradiation)**

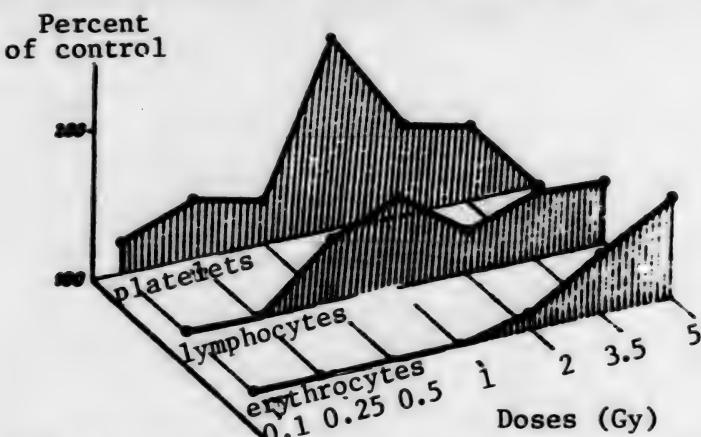


Figure 5. Comparison of ^3H -Concanavalin A Binding of Various Human Blood Cells Irradiated in Vitro in the Dose Range of 0.1-5.0 Gy and Examined 3 Hours After Irradiation.

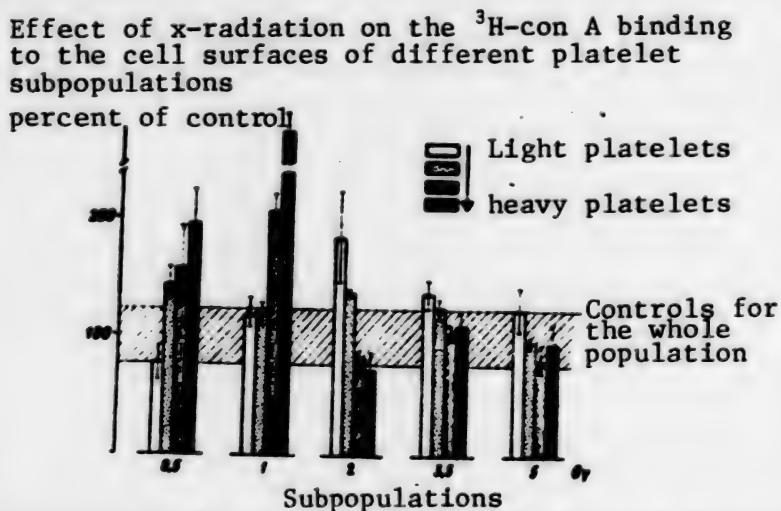


Figure 6. ^3H -Concanavalin A Binding to the Cell Surface of Various Subpopulations of Murine Platelets After X-Irradiation in the Dose Range of 0.5-5.0 Gy. The subpopulations consist of light (white), heavy (solid black), and intermediate (hatched) platelets. The columns and controls represent mean values \pm S.D.

In conclusion, the radiation-induced plasma membrane alterations as revealed by binding studies with lectin or cationized ferritin as well as by SEM are appearing early and existing temporarily; they can be detected in a wide range of mammalian cells. Therefore, the general nature of the phenomenon seems to be evident. Furthermore, reversible perturbation of plasma membrane is provoked by such low doses which do not kill the cell or do not result in irreversible destruction of the membranes. Finally, the intensity of the reaction depends on the cell-type as well as on the physiological conditions of cells, like their age.

³H-con A binding by subpopulations of human erythrocytes after x-irradiation in vitro

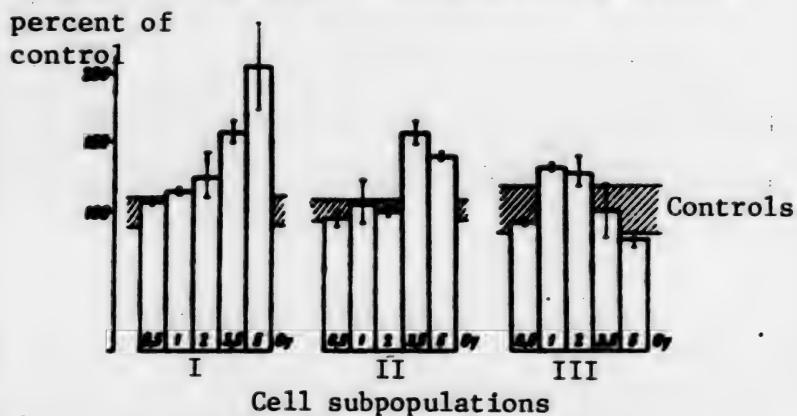


Figure 7. ³H-Concanavalin A Binding by Various Cell Populations of Human Erythrocytes Separated by Centrifugation on Ficoll-Uromiyo Discontinuous Gradient. I. light cells; II. intermediate fraction, III. heavy, slightly clumped cells. The irradiated and control data represent mean values \pm S.D.

Alterations of Virus Receptors

Having obtained the data for binding of such proteins like con A (MW 100,000) and ferritin (MW 540,000) we decided to test the receptor function of cells by much larger and more composite particles of utmost biological importance, i.e., viruses. Primary green monkey kidney cells were x-irradiated and infected with poliovirus type 2. The time period of cell-virus contacts were regulated by virus neutralization after various time periods. The end-point of infection—the appearance of cytopathogenic effects (CPE)—was checked at various days after inoculation. It can be seen in Figure 8 that 1 hour after 2.5 Gy the viruses could more easily attach and penetrate into the cells; at 4 hours the capabilities of membranes for the early events of viral infection were somewhat less than in the control, i.e. attachment and penetration of the virus were retarded.

Beside the effects of x-irradiation similar studies were performed when tritiated water (³HTO) served as radiation source. This interest was raised due to the intriguing data on radiotoxicology of ³H. Two types of tissue culture cells were tested and summary of representative experiments is shown in Figure 9. As it is seen, 1 μ Ci per ml of HTO within 10 minutes increased the lectinbinding, soon afterwards it dropped below the control level. The depression proved to be reversible as the release of cells from the effect of HTO resulted in normalization of lectinbinding. The decreased reactivities of lectin receptors were supported by scanning electron microscopic observations. A definite flattening of cells could be observed. The comparison of ³H-beta and x-irradiation effects indicated clear differences, ruffling in x-irradiated, flattening in HTO-treated cells. The virus attachment and penetration test also indicated a clear perturbation of plasma membranes.

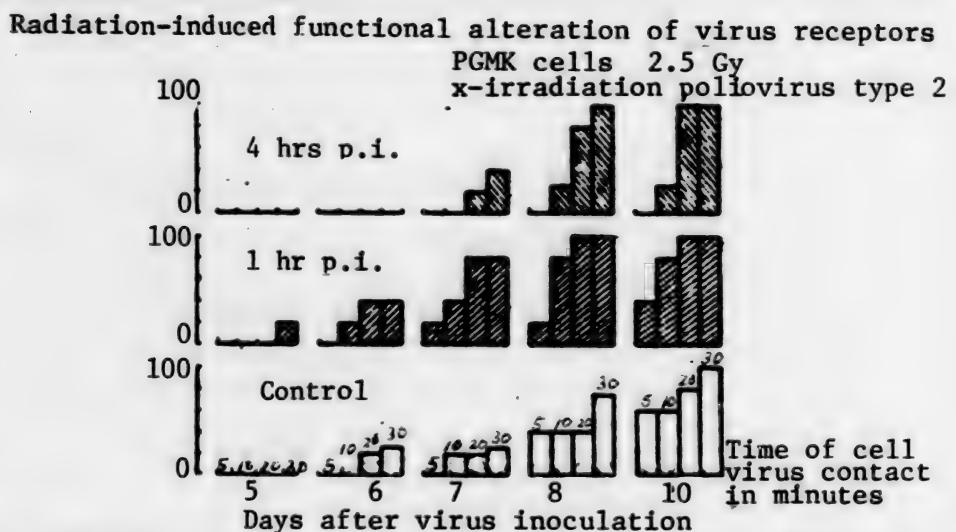


Figure 8. Radiation-Induced Functional Alteration of Virus Receptors.
Host cells: primary green monkey kidney (PGMK); virus: polivaccine type 2; x-irradiation with 2.5 Gy. The time of cell-virus contact was regulated by virus neutralization. Cytopathogenic effect (CPE) was checked at various days after virus inoculation.

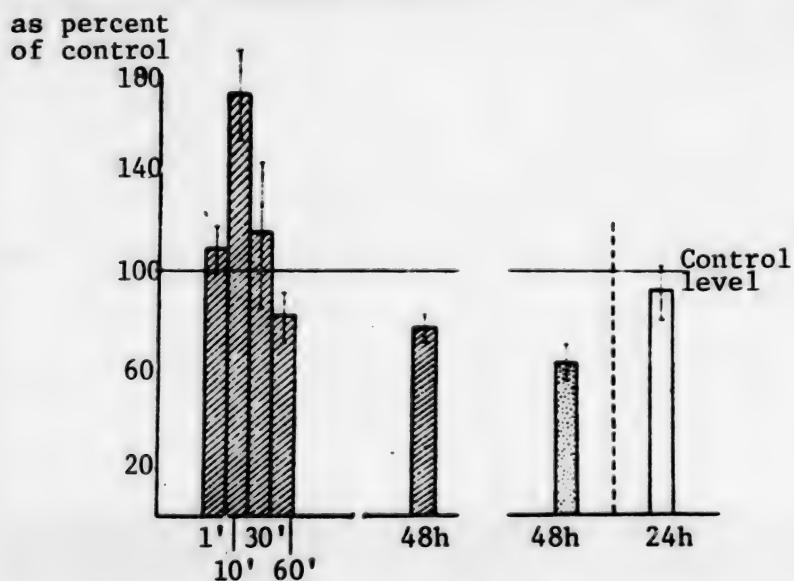


Figure 9. Effect of Tritiated Water (HTO) on ^3H -Concanavalin A Binding to Cultured Cells.
Ordinate: membrane-bound radioactivity expressed as percent of control. Abscissa: time-period of HTO-treatment with 37 kBq ($1 \mu\text{Ci}$) per ml; : primary green monkey kidney cells; : primary human fibroblasts. The cells exposed for 48 hours to HTO were transferred to HTO-free medium and examined 24 hours later, .

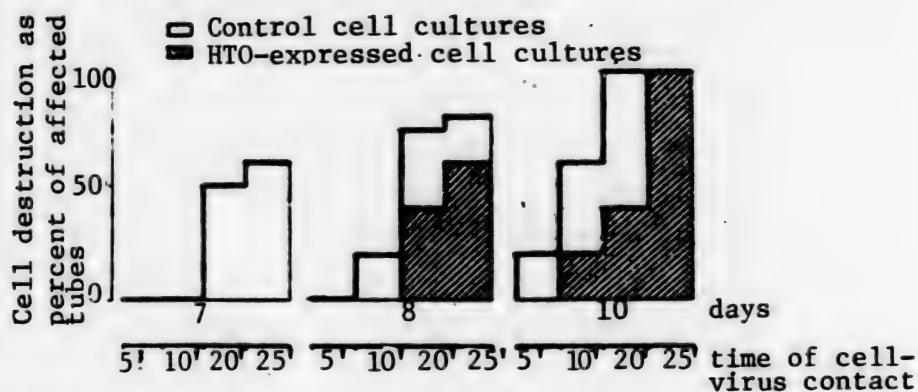


Figure 10. Development of Cytopathogenic Effect (CPE) as Function of Time of Virus Adsorption in Control Cells () and Cells Exposed to 37 kBq per ml of HTO ()

Figure 10 indicates that pretreatment of cells with HTO causes a delay of appearance of CPE. The times of virus-contact with the perturbed cell membrane were varied, when this period was kept short enough, it either delayed or prevented the attachment and penetration processes.

The results presented demonstrate that both x- and tritium-beta irradiations perturb the membrane reversibly, in such moderate dose-ranges which do not kill the cells. The directions of alterations, however, are opposite, i.e. while x-irradiation facilitated, the tritium-beta irradiation depressed the attachment and penetration of viruses. No clear explanation of this difference is available as yet.

Impact of Membrane Alterations on Various Cell Function

Although in the foregoing only some examples were given for the radiation-induced receptor alterations of the plasma membranes, it is reasonable to think that similar mechanisms may also cause the functional disturbance of the intracellular membranes. This idea is based on the similar chemical and supramolecular buildup. Therefore, through more-or-less uniform mechanisms depending on the radiation qualities and other modifying factors the nonspecific effects of radiations may result in a large variety of altered cellular processes depending on the special functions of specific membranes. There are, indeed many data accumulating which point to the alterations of nuclear⁶³ and mitochondrial⁶⁴ membranes, endoplasmic reticulum,⁶⁵ Golgi-complex.^{56,66,67} The radiobiological experience collected for plasma membranes can, therefore, be applied to the other ones, as far as the general mechanisms are concerned. It seems to be quite obvious that any penetrating radiation affects many coordinated cellular processes on multiple loci maintained by the membrane system and the fate of the cell depends on the restoration of this network. The rather quick regeneration of membrane structures and functions does not, however, exclude the possibility that during the persistence of perturbed state aphysiological events might occur even after slight radiation injuries with consequences in late phases. The "misrepair" of membrane structures

cannot be excluded either as yet. The following points are intended to emphasize the role of membranes or the conditions of membrane system on the development of regeneration of membranes or nonmembraneous radiation effects. The perturbed structural-functional units affect a series of membrane-bound cellular events and processes like permeability. This increases for ions, especially its significance for Ca^{2+} has to be mentioned,⁶⁸ which might lead to serious structural damages, as a common final pathway for interphase death was suggested through Ca-uptake.⁶⁹ Permeability also increases for fatty acids, foreign proteins, toxins. The transport of several biochemical precursors can be disturbed, e.g., the delayed uptake of glucosamine⁵⁶ and IUDR^{70,71} were reported recently. Temporary disturbance of membrane structure might lead to errors in recognition of foreign cells and particles, in membrane-mediated receptor processes of information transfer, regulation, endocytosis, infection with parasites, bacteria and viruses. Examples for the latter were presented above. The receptor function is certainly dependent on the proper constellation of surface charges as the latter are possible regulators of membrane-bound enzymes.⁷² Therefore, a temporary depolarization might result in alterations of enzyme or receptor functions. The membrane decomposition may lead to interphase death of irradiated cells if the "self-sustained" chain reaction of radical formation and consecutive lipid peroxidation last long enough in relation to the initiation and completion of regenerative processes. Combined effects due to membrane-specific drugs like procain and chlorpromazine,⁷³⁻⁷⁵ oxygen (OER)⁷⁶ and heat (TER)^{77,78} may potentiate radiation effects through membrane-related processes. Among extra-membraneous effects originated from, or connected with membrane function perturbation, several alterations were already indicated. The cell survival depends on the chemical condition of membranes; for example it decreases after enzyme digestion of cells with trypsin and neuraminidase. The membrane components or their derivates may serve as potential cocarcinogens, e.g. C-nitroso-activated form of the acrylamine carcinogens covalently binds with membrane lipid components to form a nitroxyl free radical,⁷⁹ or co-mutagens ("endogenous mutagen"), e.g. peroxidation products of lipid bilayer, malondialdehydes, can damage DNA.²⁵ The decomposition of nonmembraneous lipids, e.g., lipid peroxidation of nuclear membranes or "linkers" of DNA subunits may lead to disintegration of DNA.^{15,16,80} The partial damage of compartmentalization profoundly disturbs the membrane-regulated metabolic processes. And last but not least, it has to be realized that recovery of the injured membranes takes place at the same time as DNA repair. This simultaneous recovery and repair might influence the efficiency of repair.⁸¹ The arising problems concerning these metabolic relationships were recently reviewed.⁸²

Finally, it can be expected that the development of membrane biology and radiobiology might result in solution of several problems of practical importance. For example, a sensitive indication of radiation injury through membrane-related phenomena, stabilization of membranes and protection of membrane perturbation to prevent the temporary disturbance of their regulatory functions, the prevention of entering of noxious, chemical or biological agents the facilitation of the intracellular action of chelating compounds in decomplexation of radionuclides, the development of well-controllable artificial

carrier particles for substitution therapy, all these problems might be solved if knowledge on radiation biology of cellular membranes will grow.

As a general conclusion, the aspects of membrane radiobiology presented suggest that due to importance of the complex regulatory function of the membrane system and to the consequences of its disturbance we may expect a rapid development of the field in the near future.

The state of the art, at present, is very promising both for scientific theories and their practical applications.

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Fast-Neutron Damage to Plant Tissues

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[Article by Jadwiga H. Supniewska, Barbara Dohnal, Antonina Cebulska-Wasilewska and Jerzy Huczkowski, Polish Academy of Sciences, Institute of Pharmacology, Department of Phytochemistry, 31-343 Crakow, Poland and Institute of Nuclear Physics, 31-342 Crakow, Poland: "Biological Effects of Fast Neutron Irradiation on Callus Tissues of Tecoma Stans Juss. and Ammi Visnaga Lam"; paper received December 1981; revised November 1982.]

[Text] Callus tissues of Tecoma stans Juss. and Ammi visnaga Lam. were subjected to fast neutron irradiation. Nine doses were applied within the range of 100-10,000 cGy. Small doses caused growth stimulation. Intermediate and high doses caused morphological changes, reduced growth and biosynthesis of biologically active substances (monoterpene alkaloids in *T. stans*, furanochromones in *A. visnaga*). In *A. visnaga* neutron irradiation considerably decreased the chlorophyll content in callus tissues.

The radiosensitivity of *A. visnaga* at 50 percent growth reduction level was 1.5 times higher than that of the callus of *T. stans*. The recovery of the tissues takes place during a subculturing course. Three to 7 months after neutron exposure growth and biosynthesis reach the control level.

Introduction

Tissue culture of medicinal plants has been studied for many years and much progress has been achieved in this field, involving static and suspension culture. The main point of interest in this field is the production of secondary plant metabolites like alkaloids, cardenolides, saponines and other valuable biologically active substances. In our previous studies¹⁻³ the tissue cultures of two interesting species were investigated as a potential source of such substances: *Tecoma stans* Juss. (Bignoniaceae) which produces monoterpane alkaloids and quinones and *Ammi visnaga* Lam. (Umbelliferae) which produces furanochromones.

However, the synthesis of these compounds in tissue culture is much lower than in the intact plants and it decreases even more in the course of cultivation. Many experiments have been performed to improve the yield of these valuable substances in the cultured tissues. For this purpose various chemical and physical agents have been used: such as variations of nutrient media, precursor feeding and some changes of environmental conditions. Ionizing radiation, being widely used as a mutagenic agent for plant material⁴⁻⁷ could also have a positive effect on the biosynthesis.⁸

In this work callus tissues of *T. stans* and *A. visnaga* were irradiated with a wide range of fast neutron doses and their influence on tissue survival, growth rate and content of the secondary metabolites was examined in order to determine whether biosynthesis of these compounds could be enhanced by ionizing radiation.

Materials and Methods

T. stans callus tissue fused for irradiation was a 6-year-old strain established from the stem and stem leaves of the sterile seedlings.¹ During the last three years the tissues were subcultured on Murashige-Skoog's revised tobacco medium supplemented with kinetin, 0.3 mg/dcm³. Its growth index averaged 3.8 ± 0.3 . *A. visnaga* callus tissue strain of stem origin was cultivated for 4 years, lately on Linsmaier and Skoog's nutrient medium supplemented with kinetin, 0.1 mg/dcm³ and 2,4-D, 0.5 mg/dcm³. The tissue was developing on this medium satisfactorily and reached the 4 week growth index 2.0 ± 0.3 .

Both tissues were growing as surface, liquid cultures in Petri dishes incubated in the continuous fluorescent light of about 1,000 lux at 28°C. The tissue explants, 5 to 7 g of fresh weight of vigorously dividing tissue of the species were used for the irradiation procedure. They were transferred into empty culture tubes to avoid irradiated medium effect, and subsequently subjected to irradiation, 5 explants for every dose. The next day thus

treated tissues were removed from the tubes and placed in Petri dishes on the liquid medium for further cultivation.

The callus samples were irradiated with fast neutrons, mean energy 5.6 MeV from a nuclear reaction $^9\text{Be}/\text{d}, \text{n}/^{10}\text{B}$. Cyclotron U-120 of the Institute of Nuclear Physics in Crakow was the source of deuterons for this reaction.⁹ Dosimetry procedures have been described previously.¹⁰ Gamma contamination was 6-9 percent of the neutron dose. Doses were calculated in standard tissue. Additionally, two monitors, biological (barley seeds) and physical (sulphur pellets),¹¹ were used. Measurements of barley seedling growth under standard conditions were made in order to determine biologically the isodose curves for three different distances from the target. Since, as has previously been found,⁶ there were no significant differences in doses and in the energy spectrum in the case of simultaneous irradiation with different dose monitors only sulphur pellets were used for direct measurements of neutron doses. Several doses were applied in the range of 100 to 10,000 cGy and dose rates of 30 to 200 cGy·min⁻¹.

Morphological observations and growth measurements of the two species were performed every 4 weeks. Growth Index (G.I.) was calculated according to the formula:

$$\text{G.I.} = \frac{(\text{weight of fresh tissue}) - (\text{weight of fresh inoculum})}{(\text{weight of fresh inoculum})}$$

The samples of 10 to 20 g dry weight, collected separately from each dose during two subsequent periods after exposure, were subjected by phytochemical analysis. *T. stans* was tested for monoterpenoid alkaloid and quinone contenten using the methods described in detail earlier.² Analyses were performed twice: 6 months and 18 months after irradiation. Intensively green *A. visnaga* callus was investigated not only for furanochromone but also for chlorophyll content. The analysis of furanochromones was performed by Karawya method³ twice: first 8 weeks after irradiation in all irradiated + control samples, and 1 year after irradiation in samples irradiated with 1570 cGy dose and in control. Chlorophyll content was determined by spectrophotometric method¹² 4 weeks after exposure to fast neutrons. It was measured in acetone extracts at 660 and 642.5 nm with a spectrophotometer "Spekol" (Zeiss, Jena). The results were calculated as mg of total chlorophyll per g of dry tissue.

Results

Tecoma stans. Callus tissue subjected to irradiation consisted of homogeneous parenchymatic cells joined in nodular, predominantly compact, clumps with a few softer fragments, randomly distributed. The tissue was slightly grey with an olive-green shade and showed a good biomass increase. This tissue strain was proved to biosynthesize: (a) three monoterpenoid alkaloids characteristic for this species: tecomanine, actinidine and one not clearly identified, skyanthine derivative, (b) a quinone compound: lapachol.² However, the ability to biosynthesize the last metabolite had been lost in the course of cultivation in vitro.

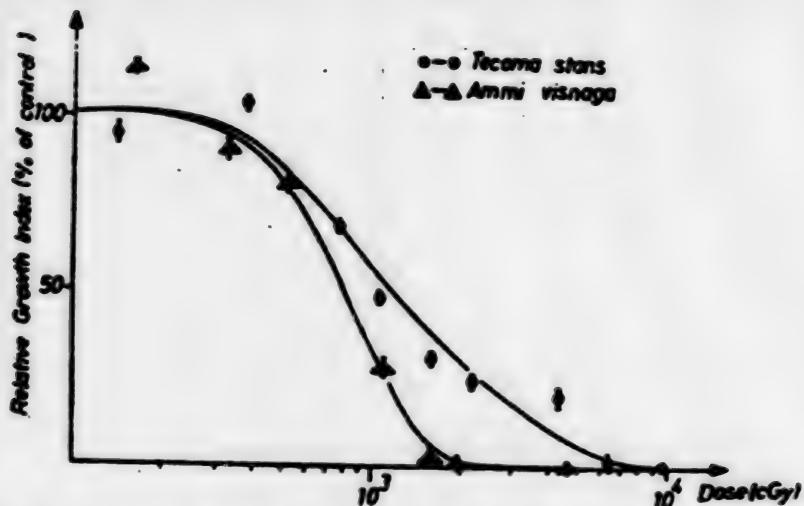


Figure 1. Dose-Dependent Growth of Neutron Irradiated *Tecoma stans* and *Ammi visnaga* Callus Tissues 4 Weeks After Exposure

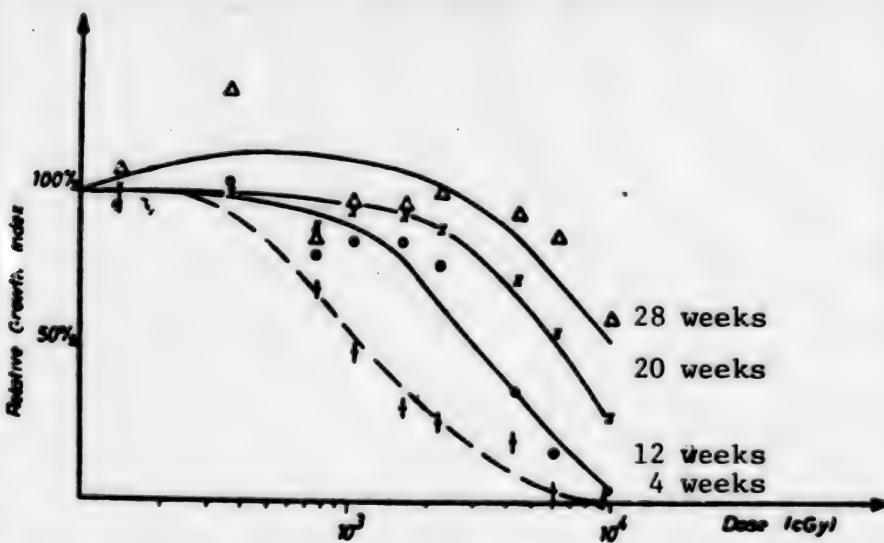


Figure 2. Dose-Dependence of Growth Rate of *Tecoma stans* Callus Tissues, Measures at Different Time of Subculturing After Exposure to Neutron Irradiation

The observations of morphological changes and the measurements of biomass gain performed 4 weeks after irradiation showed three types of radiation responses. Up to 800 cGy there was a minute reduction or for some doses, a small stimulation of the growth rate. Morphologically the irradiated tissue looked like the control one. Tissue irradiated with 1,000 to 4,000 cGy dose showed a significant growth rate reduction and the callus more compact and consisting of firm cell aggregates, turned partly brown or sometimes even necrotic. The tissue irradiated with doses higher than 4,000 cGy displayed very large injuries. Just at 4,000 cGy dose growth inhibition reached about 80 percent and the whole tissue became brown. Above 6,000 cGy dose the tissues became necrotic and black. The results of the tissue growth measurements are shown in Figures 1 and 2.

Further observations showed that 2 to 3 months after irradiation on the almost completely dead tissues (over 6,000 cGy dose) there appeared a few small and soft clumps of young, whitish-grey callus that continued to develop. The growth measurements performed in subculturing showed that up to half a year after neutron treatment all the damaged tissue improved their growth-rate and reached the control level with the rate depending on the degree of injury (Figures 2 and 3). Afterwards no significant differences were observed.

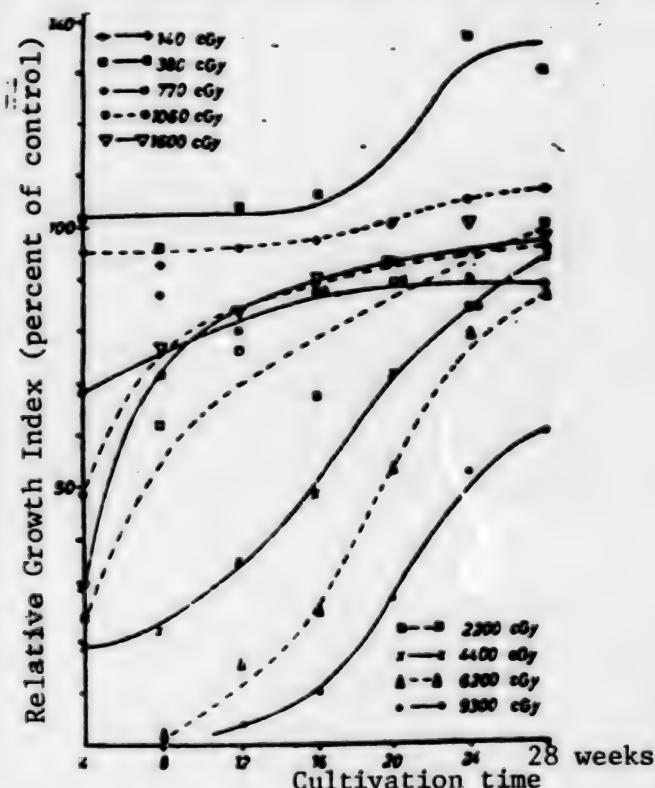


Figure 3. Influence of the Cultivation Time on *Tecoma stans* Growth Inhibition Caused by Different Doses of Neutron Irradiation.

Table 1. Alkaloids Revealed by Chromatography (TLC) in Control and Fast Neutron Irradiated Callus Tissues of *T. stans*

Alkaloids	Callus tissues collected	After irradiation			
		Control	100-800 cGy	6th month 1,000-4,000 cGy	From the 7th-18th month 4,000-10,000 cGy
Actinidine		++	++	-	-
Tecomanine		++	++	+	-
Skytanthine derivative		+++	+++	++	+

+++ spots very pronounced

++ spots well marked

+ traces

- no spots

Thin layer chromatography of the tissue extracts demonstrated (Table 1) that the content of alkaloids at 6 months after irradiation declined with the increasing radiation dose. This radiation response was similar to that of the response of the growth rate to neutron exposure. In irradiated calluses no actinidine and tecomanine could be detected when the radiation dose was at the level of 1,000 cGy and 4,000 cGy, respectively. After next 12 months of subculturing the alkaloid spectrum in all tissue samples was the same and nearly equal to the control (Table 1).

A slight stimulation tendency in tissue growth-rate was not accompanied by any increase in biosynthesis of metabolites. In all irradiated tissues no lapachol could be detected.

Ammi visnaga. The experiment was performed on the homogeneous, green and fine callus tissue strain. During 4 years of cultivation the strain was able to produce furanochromone-visnagin but lost the ability for furanocoumarin-marmesin biosynthesis.³ Detailed observations and measurements of the tissue responses were performed 4 weeks after irradiation.

Starting from about 500 cGy dose neutron exposure caused evident dose-dependent reduction of tissue growth (Figures 1 and 5), and decline in chlorophyll content (Figure 4). The dose of 1,570 cGy entirely stopped the tissue growth. At higher, lethal doses tissue culture was not capable of efficient regeneration. Tissues irradiated with doses lower than 1,570 cGy started to regenerate and their development in 6 months after exposure equalled the control one. The lowest dose, viz. 160 cGy, did not cause any inhibition but showed very slight stimulation tendency, that resulted in growth increase equal to about 110 percent of the control.

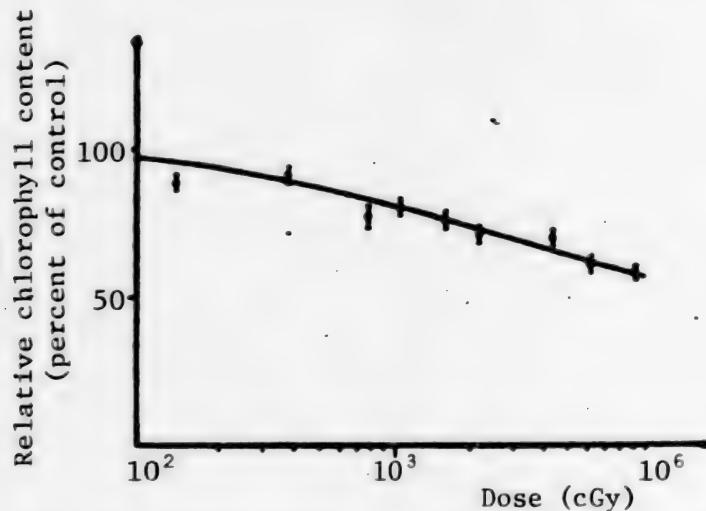


Figure 4. Neutron Dose-Effect Curve of Chlorophyll Content in *Ammi visnaga* Callus Tissue



Figure 5. Tissue Culture of *Ammi visnaga* 4 Weeks After Irradiation.
K: control; 9: 160 cGy; 7: 520 cGy; 5: 1,570 cGy;
3: 4,670 cGy; 1: 9,000 cGy.

The chlorophyll response was manifested by fading of the tissue color, which with the increasing neutron doses became pale-green. Starting from about 1,000 cGy dose the tissue put on a yellow shade and the necrotic tissue over 5,000 cGy dose turned dark brown. Besides, anatomical observations showed an interrelation between chlorophyll loss and the size of chloroplast. Disc-like chloroplasts in the control cells reached about 50 μ in diameter, whereas those in the irradiated tissues were much smaller, from 15 μ to the minute grains in the necrotic tissues. The chlorophyll content in irradiated tissues evidently lowered and at extremely large doses declined to 60 percent of the control value. After 3 to 7 months, depending on the degree of injury, chlorophyll content and the size of chloroplasts returned to the control level.

The comparative examination by thin layer chromatography showed the appearance of one spot of furanochromone-visnagin in the tissue extracts of control and in samples which were irradiated with the doses from 160 cGy to 1,570 cGy of fast neutrons. The spots of visnagin originating from comparable samples of irradiated tissue were smaller and less distinct than that from the control tissue. This suggests that neutron exposure restrains biosynthesis of furanochromone. One year after exposure the analysis of furanochromone content in control and in irradiated with a dose of 1,570 cGy tissues showed no difference in the size and in the intensity of the spots of visnagin. No other furanochromone or furanocoumarine (marmesin) spots were detected in thin layer chromatography of these samples. This means that after recovery of biological balance the synthesis of secondary metabolites returned to its normal level and no stimulation of biosynthesis was observed.

Discussion

Based on the shown responses of *Tecoma stans* Juss. and *Ammi visnaga* Lam. callus tissues to ionizing radiation it may be concluded that the fast neutrons affect the growth, morphology and biosynthesis of metabolites of the two species and that the biological effects are to some extent proportional to the radiation dose.

A comparison of the ionizing radiation response of *T. stans* and *A. visnaga* callus tissues showed that the second species is more sensitive to fast neutron irradiation. At the 50 percent growth level *A. visnaga* shows the radio sensitivity 1.5 times higher (Figure 1) than *T. stans* does.

One of the observed biological effects was a progressive growth depression, leading to the necrotic changes above 1,500 cGy and 6,000 cGy doses in *A. visnaga* and *T. stans*, respectively. However, low doses, 160 cGy (*A. visnaga*) and 380 (*T. stans*), tend to stimulate growth slightly. Debulska-Wasilewska, et al.,¹³ and Miszko, et al.,⁶ reported similar results and interpreted them as a consequence of post-irradiation disturbances in hormonal level of the callus tissues. Kryukova and Maevskaya¹⁴ and Kuzin, et al.,¹⁵ showed that low doses of ionizing radiation increased the kinetin and gibberellin levels in plants. This was followed by activation of protein and RNA synthesis, and in consequence, stimulated plant growth, whereas higher doses diminished the hormonal level, thus causing growth inhibition. Although high doses caused death in the majority of *T. stans* callus cells in 2 to 3 months, some number of new not numerous, tissue sprouts regenerated on the surface of the necrotic tissue. Proliferation in lethally irradiated organs may start from a radiation resistant subpopulation of cells and/or from other cells that have recovered from radiation injury and initiated divisions at a later time.¹⁶

Regeneration of lethally irradiated callus was not observed in a case of *A. visnaga*. This indicates that callus tissue cells of *A. visnaga* are more homogeneous in respect to their radio sensitivity than the cells of *T. stans*.

The growth reduction is accompanied by an inhibition in biosynthesis of biologically active substances in both species, monoterpene alkaloids in *T. stans* and furanochromones in *A. visnaga*. At higher doses production of these metabolites diminished significantly.

Lapachol biosynthesis in *T. stans* and marmesin in *A. visnaga* calluses lost in cultivation course before irradiation was not restored by neutron treatment. The ionizing radiation failed also to give rise to alkaloid synthesis in *Tylophora indica* callus strain, which had earlier lost this ability.⁴ However, in the tissue exposed to 2,000 cGy dose the authors found phenolic compound, quercetin which was not recorded in this tissue strain before.

Miszke, et al.,⁶ assumed that beside damaging growth substances, ionizing radiation also causes some disturbances in the biosynthesis of enzymes and consequently in the biosynthesis of the respective metabolites. The decrease or a complete lack of these substances in the callus tissue might then result from the radiation-induced injury of the enzyme system involved.

Restoration of biological equilibrium in irradiated tissues is accompanied by normalization of the growth and metabolites production rates. No overproduction of secondary metabolites was observed in tissues in which there was radiation-stimulated growth. However, the method of analysis used in this work (thin layer chromatography) is of low precision to find whether production of secondary metabolites is increased some 10-20 percent by low doses of radiation. The stimulation of the production of secondary metabolites might probably occur as a result of radiation-induced mutation changes in some callus cells, which could develop more efficient cell lines. A direct stimulation of a biosynthetic apparatus might also operate in irradiated tissues. The application of more accurate methods of phytochemical analyses and cell cloning followed by selection of promising strains will improve the further investigations into the question of the mechanisms and expectancy of radiation-increased production of secondary metabolites in callus tissues grown in vitro.⁸

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